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GROWTH OF A STRAIN OF *SALMONELLA ENTERITIDIS* EXPERIMENTALLY INOCULATED INTO CANNED FOODS[1]

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Certain species of *Salmonella* have been held as causative agents of food poisoning. Although these organisms have been studied in animal hosts and in culture media, practically no study has been made of their growth in foods. In this investigation a study has been made of the growth of a strain of *Salmonella enteritidis* inoculated into canned foods. Representative canned foods were chosen for these experiments, including tomato juice and peaches (acid type); asparagus, spinach, and string beans (semiacid type); peas and corn (low-acid type); shrimp and salmon.

EXPERIMENTAL PROCEDURE AND RESULTS

A stock culture of *S. enteritidis*, isolated in 1914 from the stool of a patient who was taking care of calves during an epidemic of enteritis, was grown in veal infusion broth for six to 24 hours and the broth culture, or a dilution thereof, was inoculated directly into the various cans of foods. The technique of handling the cans has been previously described by Segalove, Davison, and Dack (1943). The total number of organisms inoculated per can of food was determined by plate counts. The number of organisms inoculated per milliliter of fluid contents was calculated by dividing the total count of the inoculum by the total number of milliliters capacity of the can. Eight cans of each food were always inoculated. Four were incubated at 37°C.(98.6°F.) and four at 22°C.(71.6°F.).

Plate Counts: At definite time intervals the cans were removed from the incubators, shaken, and samples taken. Decimal dilutions were made of the liquid sample in sterile 0.85 per cent salt solution. Veal infusion agar pour plates were made with one-ml. portions of the decimal dilutions. After 48 hours' incubation at 37°C. the colonies were counted.

Bacterial counts of each sample were made after 2, 7, 14, 30, and 60 days' incubation as indicated (Table 1).

At 22°C. the strain of *S. enteritidis* used in these experiments grew in all the canned foods tested except peaches. At 37°C. no growth occurred in peaches or asparagus (Fig. 1 and Table 1).

Gas developed in canned peas, corn, asparagus, and string beans (Table 2). This was variable, depending upon the kind of food and incubation temperature. More gas was produced in canned peas at both incubation temperatures than in corn at 22°C. or in asparagus and string beans at both temperatures. Peas incubated at both 22 and 37°C. and corn incubated at 37°C. produced enough gas to cause the ends of the

[1] This work was aided by a grant from the National Canners Association.

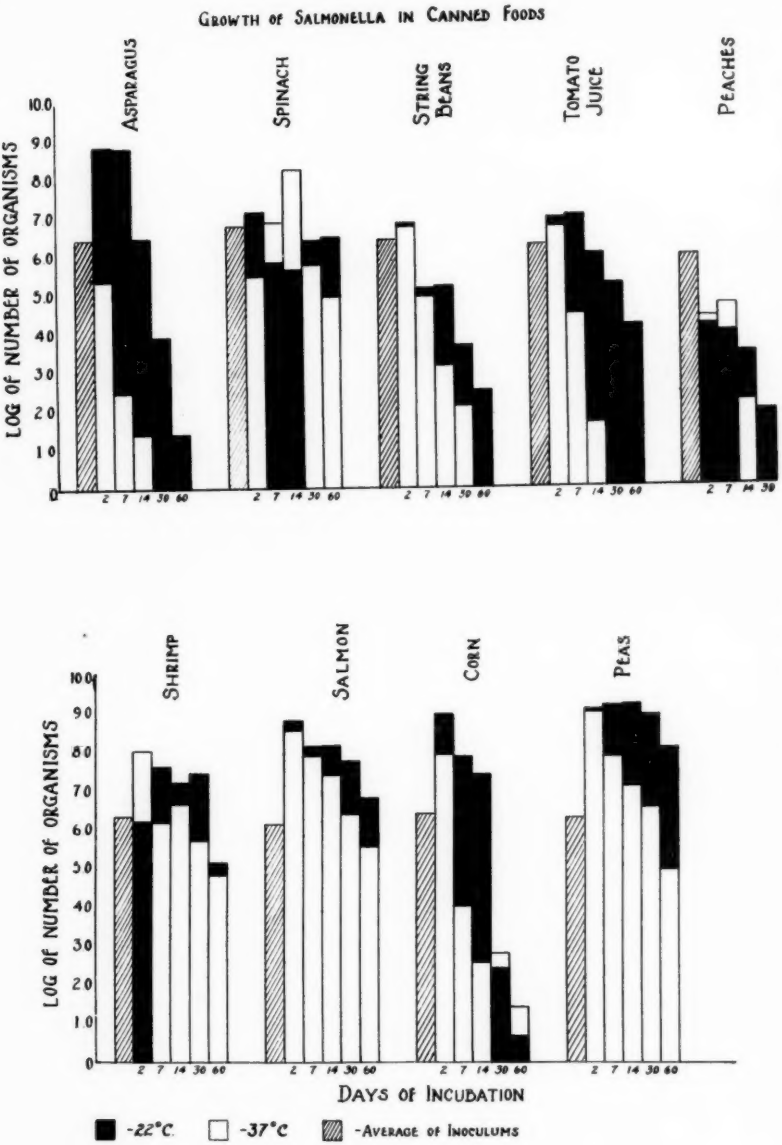

FIG. 1

TABLE 1

Individual Counts on Each Food Inoculated With Salmonella enteritidis

TABLE 1

Individual Counts on Each Food Inoculated With Salmonella enteritidis

Series	Canned food	Number of organisms inoculated per ml. of fluid contents [1]	Number of organisms per ml. of fluid contents after incubation at									
			22°C.(71.6°F.)					37°C.(98.6°F.)				
			2 days	7 days	14 days	30 days	60 days	2 days	7 days	14 days	30 days	60 days
1	Asparagus	3×10^6	233×10^6	337×10^6	65×10^5	19×10^3	100	36×10^4	$<10^2$	$<10^2$	0	0
2	Asparagus	2×10^6	332×10^6	86×10^6	10×10^4	13×10^2	1	1×10^4	100	0	0	0
3	Asparagus	2×10^6	125×10^6	21×10^7	30×10^5	124×10^2	4	91×10^4	100	0	0	0
4	Asparagus	4×10^6	87×10^7	189×10^7	24×10^5	370	0	80×10^5	<10	0	0	0
1	Spinach	3×10^6	285×10^5	8×10^5	140×10^4	452×10^4	296×10^2	3×10^5	35×10^5	$>10^5$	42×10^4	346×10^3
2	Spinach	1×10^6	26×10^5	145×10^4	32×10^4	261×10^4	280×10^4	$>10^4$	186×10^4	110×10^4	188×10^4	28×10^2
3	Spinach	2×10^6	43×10^5	$<10^4$	100	$>10^3$	95×10^3	89×10^4	51×10^5	69×10^7	2×10^3	8×10^3
4	Spinach	3×10^6	194×10^5	39×10^4	$>10^4$	235×10^4	$>10^4$	6×10^4	190×10^5	$>10^4$	40×10^4	8×10^2
1	String beans	2×10^6	237×10^4	2×10^4	186×10^2	100	0	108×10^4	$<10^5$	$<10^2$	61	0
2	String beans	2×10^6	245×10^4	109×10^3	271×10^2	20	1	130×10^5	155×10^3	6×10^2	0	0
3	String beans	2×10^6	103×10^5	12×10^4	33×10^4	173×10^2	10	20×10^5	7×10^3	250	0	0
4	String beans	4×10^6	114×10^5	31×10^4	27×10^4	17×10^3	Contam.	54×10^5	16×10^4	48×10^2	450	0
1	Tomato juice	2×10^6	178×10^4	322×10^4	265×10^4	44×10^4	26×10^2	93×10^4	50×10^3	$<10^2$	0	0
2	Tomato juice	1×10^6	90×10^4	78×10^4	122×10^4	22×10^4	437×10^2	131×10^5	28×10^3	60	0	0
3	Tomato juice	2×10^6	150×10^5	192×10^5	75×10^3	233×10^2	93×10^2	50×10^3	87×10^2	10	0	0
4	Tomato juice	3×10^6	156×10^5	185×10^5	76×10^3	86×10^2	33×10^2	48×10^4	31×10^3	3	0	0
1	Peaches	9×10^5	78×10^3	940	33	0	0	186×10^3	70	1	0	0
2	Peaches	9×10^5	254×10^3	36×10^3	108×10^2	11	0	84×10^3	190×10^3	520	0	0
3	Peaches	9×10^5	99×10^3	15×10^2	122	3	0	11×10^2	$<10^2$	0	0	0
4	Peaches	9×10^5	141×10^3	182×10^2	290	320	0	$<10^2$	0	0	0	0
1	Shrimp	3×10^6	30×10^5	123×10^6	89×10^5	53×10^5	92×10^3	109×10^5	72×10^5	60×10^4	41×10^2	93×10^2
2	Shrimp	1×10^6	$<10^4$	406×10^5	52×10^5	7×10^5	180×10^3	254×10^5	98×10^5	87×10^4	590	$<10^2$
3	Shrimp	2×10^7	11×10^5	93×10^5	59×10^5	31×10^4	144×10^3	165×10^5	260×10^5	76×10^4	500	10×10^4
4	Shrimp	3×10^6	272×10^4	3×10^4	49×10^6	114×10^5	14×10^4	37×10^5	224×10^5	170×10^5	205×10^4	15×10^4
1	Salmon	2×10^6	75×10^7	108×10^6	364×10^6	110×10^6	42×10^5	228×10^6	117×10^6	271×10^6	254×10^4	45×10^3
2	Salmon	8×10^5	41×10^7	105×10^6	59×10^6	45×10^5	199×10^5	444×10^6	124×10^6	287×10^5	293×10^4	125×10^4
3	Salmon	1×10^7	120×10^7	372×10^6	52×10^6	29×10^6	52×10^5	81×10^7	50×10^6	20×10^6	127×10^4	250×10^3
4	Salmon	2×10^6	30×10^7	203×10^5	145×10^6	168×10^6	30×10^4	$<10^5$	264×10^5	27×10^6	38×10^5	$>10^2$
1	Corn	1×10^6	18×10^6	276×10^5	3×10^5	$<10^2$	<10	36×10^7	<20,000	<1,000	25×10^2	<10
2	Corn	6×10^5	274×10^6	47×10^5	95×10^6	600	6	$>10^7$	<2,000	400	<20	42
3	Corn	8×10^6	352×10^7	$>10^5$	504×10^4	110	0	8×10^6	<20,000	200	120	0
4	Corn	2×10^6	218×10^6	160×10^6	190×10^5	$>10^2$	Contam.	20×10^5	<200	<10	<10	0
1	Peas	2×10^6	512×10^6	76×10^7	391×10^6	354×10^6	160×10^6	399×10^6	168×10^6	118×10^5	76×10^5	36×10^5
2	Peas	1×10^6	62×10^7	175×10^7	200×10^7	144×10^7	79×10^5	68×10^7	110×10^6	60×10^5	$<10^4$	$<10^4$
3	Peas	2×10^6	176×10^7	165×10^7	198×10^7	143×10^7	297×10^6	107×10^7	30×10^6	25×10^6	89×10^5	306×10^3
4	Peas	4×10^6	27×10^6	283×10^7	323×10^7	78×10^7	79×10^6	151×10^7	24×10^6	178×10^6	$<10^5$	$<10^3$

[1] Size of inoculum = one ml.

cans to bulge. Only slight amounts of gas were noted in canned asparagus incubated at 22 and 37°C. and in canned string beans and corn incubated at 22°C. No gas was present in string beans incubated at 37°C.

The cause of gas production in canned asparagus incubated at 37°C. for two days has not been established. A possible explanation may be due to growth of *Salmonella* which was not detected. Although no growth of *Salmonella* was evident after two days' incubation, it is possible that growth might have occurred during the early part of this period with subsequent death of many cells by the time of testing. After the two-day incubation period no further gas was produced.

TABLE 2

Production of Gas in Canned Food Experimentally Inoculated With Salmonella enteriditis

Food	Incubated at 22°C.	Incubated at 37°C.
Asparagus	+	+
Spinach	0	0
String beans	+	0
Tomato juice	0	0
Peaches	0	0
Shrimp	0	0
Salmon	0	0
Corn	+	+
Peas	+	+

DISCUSSION

One striking feature in this study of canned foods is that more growth of *S. enteritidis* was obtained at 22°C. than at 37°C. In only a few instances was the bacterial count higher in the cans incubated at 37°C. (Fig. 1 and Table 1). In similar experiments with *Staphylococcus aureus*, Segalove, Davison, and Dack (1943), growth was also found better at 22°C. than at 37°C.

The fluid portion of the canned goods was tested after the cans had been thoroughly shaken. Had the solid particles of food been tested the counts might have been different.

Although the natural habitat of *S. enteritidis* is the intestine of certain animals, particularly rodents, this organism is able to multiply under various conditions outside of the animal body. However, under these experimental conditions it has been shown to grow in certain canned foods of plant and animal origin. Although in its natural habitat the organism is accustomed to body temperature it appears to grow better and persist longer at 22°C. This is evident (Fig. 1) and it may be observed that, in general, the black areas representing growth at 22°C. in most instances exceed the height of the white areas which represent the growth at 37°C. Furthermore, in the case of asparagus, string beans, tomato juice, and peaches viable organisms were found at 22°C. after 30 or 60 days, whereas at 37°C. no viable organisms could be detected in these same foods at 30 days with the exception of string beans. Obviously, *Salmonella* are not found in unopened canned foods since the

processing times and temperatures of canning preclude the survival of this organism. *Salmonella* food poisoning, therefore, could only occur from canned foods after opening and contamination with the specific organism.

Since food poisonings owing to *Salmonella* are rare, it is obvious that these organisms do not survive for any length of time outside of their natural hosts. This is in sharp contrast to the common occurrence of *Staphylococcus* food poisoning outbreaks which are probably due to the ubiquity of the *Staphylococcus* in nature.

SUMMARY

A strain of *S. enteritidis* was experimentally inoculated in cans of asparagus, spinach, string beans, tomato juice, peaches, shrimp, salmon, corn, and peas. Duplicate cans of each food were incubated at 22 and 37° C. Growth occurred in asparagus, peas, corn, spinach, string beans, tomato juice, shrimp, and salmon incubated at 22°C. and in peas, corn, spinach, string beans, tomato juice, salmon, and shrimp at 37°C.

The significance of these findings are discussed in relation to food poisoning.

REFERENCE

SEGALOVE, M., DAVISON, E., AND DACK, G. M., 1943. Growth of a food-poisoning strain of *Staphylococcus* experimentally inoculated into canned foods. Food Research 8, 54-57.

ACTIVATION OF PAPAIN DURING DIGESTION OF MEAT[1,2]

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Enzymatic digests of meat were prepared over a generation ago by Marcano (1891) and recommended by him for the diet of invalids. At present such digests are of special interest because they can be used in dehydrated form. We have prepared meat digests by the action of papain at 70°C.(158°F.). These digests can be dried and sterilized more readily than undigested meat. The fat in the dried sample does not quickly become rancid. It is the purpose of this paper to describe the activation of papain which takes place during this digestion.

Papain can be inactivated by oxidizing agents, such as hydrogen peroxide, and reactivated by cyanide and by SH compounds, such as cysteine, Purr (1935) found that papain can also be partially activated by treatment with washed muscle and attributed this activation to the fixed SH groups of the muscle proteins. The present experiments show, however, that when inactive or nearly inactive papain is used in the digestion of such varied materials as beef muscle, liver, and new beer, the enzyme becomes progressively more active as the proteolysis proceeds, and hitherto covered sulfhydryl groups are exposed. It follows that some proteins may be digested without difficulty by papain that was initially inactive. Furthermore the original activity of the enzyme as determined by the usual methods gives no indication whatever of its potency in digesting such substrates.

Papain that clots milk or digests hemoglobin very slowly, unless activated by cyanide, can digest meat rapidly without cyanide activation (Table 1). It may be noted in Table 1 that all three samples of papain behaved alike in digesting beef, although their behavior with hemoglobin and with milk showed that two of them were practically inactive toward latter substrates unless pretreated with cyanide. When previously inactive papain has been in contact with meat it can thereafter clot milk (Table 2) and digest hemoglobin (Table 3). Table 2 shows that more papain was activated in one hour at 70°C. than in 24 hours at 23°C.(73.4°F.). At 23°C. papain digests meat more slowly than at 70°C. The more rapid activation at 70°C. is probably caused by the uncovering of a greater number of SH groups as the beef is digested. Similar activation is shown (Table 3) where the action has been measured by the rate of tyrosine liberation. In this experiment three-quarters as much digestion was done by papain paste that had been completely oxidized with H_2O_2 as by the same quantity of unoxidized, fully active enzyme.

[1] Enzyme Research Laboratory Contribution No. 86.

[2] This work was done under a Bankhead-Jones basic research project supported by special research funds.

[3] Now with the War Department.

TABLE 1

Activity Measurements of Untreated, Activated, and Inactivated Papain

Samples	Milk-clotting activity		Hb. digesting activity		Beef digestion	
	Clotting units per gm. papain with CN	Per cent without CN	Hb. units per gm. papain	Per cent without CN	Color value[1] with CN	Per cent without CN
A commercial sample of papain	300	10	14	2	282	77
Papain paste	385	87	19	42	331	78
Papain paste + H_2O_2	100	0	7	0	168	88

[1] Expressed as milligrams of tyrosine split from 100 grams of beef muscle by 40 milk-clotting units of papain at 70°C. ± 5° in three hours.

TABLE 2

Rate of Activation of Papain During Digestion of Beef at 23 and 70°C.(73.4 and 158°F.)

Temperature	Time	Milk-clotting units per c.c. of filtrate[1]			
		Commercial papain		Papain paste + H_2O_2	
		No CN	CN	No CN	CN
°C.	hr.				
23	0.0[2]	0.3	2.1	0.0	0.3
	0.5	1.5	....	0.4	....
	1.0	1.4	....	0.5	....
	2.0	1.0	....	0.5	....
	24.0	1.9	....	0.9	....
70	0.0[2]	0.3	2.1	0.0	0.3
	0.5	2.2	....	0.5	....
	1.0	3.0	....	1.5	....
	2.0	2.5	....	1.2	....
	24.0	0.3	....	0.2	....

[1] One gram of papain was diluted to 50 c.c., and 10 c.c. of the suspension was used in each test, together with 10 grams of ground beef muscle. The mixtures were filtered after incubation and the milk-clotting test carried out on the filtrate. The time recorded shows when filtration of the digest began. As a rule filtration required 10 to 15 minutes more time. [2] Indirect determination by assay of original enzyme suspension.

TABLE 3

Rate of Digestion of Beef at 68°C.(154.4°F.) by Papain Paste and by Peroxide-Treated Papain Paste[1]

Time of digestion	Color value (expressed as mg. tyrosine) formed in the digestion of 100 gm. of ground beef		
	(A) With untreated papain paste	(B) With H_2O_2-treated papain paste	(B)/(A)
hr.			
1	115	86	0.75
2	224	165	0.74
3	310	242	0.78

[1] Ten grams of ground beef were digested with 10 mg. of the papain preparation in 10 c.c. of water at 68°C. The mixtures were then cooled in ice, trichloracetic acid was added, and the phenol value of the filtrates determined as usual, Folin and Ciocalteau (1927).

These results show that inactive papain is activated by meat during digestion, and that the addition of an activator to enzyme used in such a case is unnecessary. Papain that has been inactivated by hydrogen peroxide may also be reactivated by brief treatment with beef muscle digested with pepsin and thereafter heated to destroy that enzyme. Such peptic digests give a strong test for SH with nitroprusside and lose this property together with that of activating papain on exposure to the air for several days.

The activator occurring naturally with the papain enzymes is not necessarily involved, for the activation of a crystalline papainase, chymopapain, also takes place rapidly after the addition of meat digested with pepsin (Table 4).

It is apparent that the reactivation of papain by muscle tissue is brought about, in large part at least, by SH groups in the meat. Purr (1935) also reached this conclusion when working with washed muscle. The participation of such groups is indicated by the observation that meat already disintegrated by pepsin and then treated with iodoacetamide until it no longer gave a nitroprusside test, was also no longer able to reactivate papain (Table 4). The iodoacetamide in these experiments did not affect the enzyme itself, for reactivation resulted when a mixture of treated and untreated peptic digests was used.

Hemoglobin solutions, unlike meat, do not contain a strong activating system. They may even contain heavy metals which cause some inactivation of the enzyme. The rate at which a given sample of papain digests hemoglobin is therefore no measure of the power of the sample to digest meat. Similarly, the rate of milk-clotting is no measure of the potential proteolytic activity, because the milk clots before the protein is digested to any great extent. If, however, the papain is first activated by cyanide, the rate at which this cyanide-activated papain clots milk or digests hemoglobin is a rough measure of the power of the untreated papain to digest meat.

These results are not peculiar to meat. Papain is used to chill-proof beer and the results (Table 5) show that beer, like beef muscle, can partially reactivate completely oxidized papain. The identical results obtained with filtered and unfiltered beer, and with beer prepared from either rice grits or corn, indicate that activation is probably brought about by soluble material.

EXPERIMENTAL PROCEDURE

Salt-paste papain from Hawaii, employed by Balls, Lineweaver, and Schwimmer (1940), was used as a suspension containing two grams in 100 c.c. of air-free water. This suspension was inactivated by the addition of about 0.3 c.c. of 30-per cent H_2O_2 at room temperature. Commercial papain was employed as a filtered aqueous extract. Crystalline chymopapain [4] was oxidized with the minimum quantity of H_2O_2. Commercial papain was extracted with distilled water, filtered, and otherwise treated like papain paste.

[4] The author is indebted to Bernard Axelrod of this laboratory for the preparation of crystalline chymopapain.

TABLE 4

Activation of Crystalline Chymopapain by Meat Digested With Pepsin, and Prevention of Activation by Iodoacetamide

Treatment of enzyme	Time	Milk-clotting units per c.c. of mixture	
		Without CN	With CN
	min.		
Untreated	....	0.63 [1]	3.0 [2]
Treated with H_2O_2	....	0.0 [1]	1.7 [2]
Treated with H_2O_2 + 12 c.c. peptic beef digest [2]	2	1.2	3.0
	10	2.0	3.0
	30	2.7	3.5
	45 hr.	2.4	4.0
Treated with H_2O_2 + 12 c.c. peptic beef digest treated with iodoacetamide	2	0.0	2.3
	10	0.0	2.4
	30	0.0	2.2
	45 hr.	0.0	1.5
Treated with H_2O_2 + 6 c.c. peptic beef digest + 6 c.c. peptic beef digest treated with iodoacetamide	2	1.3	2.8
	10	1.7	2.9
	30	2.0	2.7
	50	2.0	3.1
	45 hr.	0.0	1.8

[1] Calculated from assays of the respective enzyme solutions. [2] Peptic beef digest was prepared as described later and the pH adjusted 5.7. Fifty c.c. of this digest was brought to pH 7.5 with NaOH, and 0.1 *M* iodoacetamide was carefully added until a negative nitroprusside test was obtained with material that had stood one hour. The pH was then brought to 5.5 with HCl. The additions of liquid lowered to concentration of beef solids to about one-half the amount in the original pepsin digest.

TABLE 5

Activation of Papain by Beer

Papain sample	Beer sample	Time	Milk-clotting units per c.c.	
			Without CN	With CN
		min.		
Crystalline chymopapain [1]	.....	....	15.0	35.0
Crystalline chymopapain + H_2O_2	.....	....	1.0	22.0
Crystalline chymopapain + H_2O_2	Unfiltered beer	10	7.5	.....
		30	9.9	.....
		50	9.7	.....
Crystalline chymopapain + H_2O_2	Filtered beer	10	6.8	.....
		30	9.2	.....
		50	9.9	.....
Papain paste [2]	.....	....	10.4	15.0
Papain paste + H_2O_2	.....	....	0.0	6.9
Papain paste + H_2O_2	Grits, closed vat	3	1.3	.....
Papain paste + H_2O_2	Grits, closed vat	24	1.6	.....
Papain paste + H_2O_2	Grits, open vat	3	1.5	.....
Papain paste + H_2O_2	Grits, open vat	24	1.6	.....
Papain paste + H_2O_2	Rice, closed vat	3	1.5	.....
Papain paste + H_2O_2	Rice, closed vat	26	1.5	.....
Papain paste + H_2O_2	Rice, closed vat	19 hr.	1.3	.....
Papain paste + H_2O_2	Rice, open vat	3	1.6	.....
Papain paste + H_2O_2	Rice, open vat	24	1.5	.....
Papain paste + H_2O_2	Rice, open vat	19 hr.	1.4	.....

[1] In the experiments with chymopapain 0.5 c.c. of enzyme solution was added to 4.5 c.c. of beer (or water) and the milk-clotting test carried out on one c.c. of the mixture. The sample of filtered beer had been held in the refrigerator at 5°C.(41°F.) and filtered at that temperature. [2] In experiments with papain paste, 15 c.c. of beer were added to two c.c. papain solution (two gm. in 50 c.c. H_2O) in test tubes. One c.c. of the mixture was used for the milk-clotting test.

Milk-clotting tests for papain activity were carried out at 40°C. (104°F.) with 10 c.c. of buffered Klim, according to Balls and Hoover (1937). When the papain preparations were activated for this test, one c.c. of papain solution was treated for three minutes with 2 *M* NaCN. Oxidized papain paste was activated with 10 drops of cyanide solution, all other papain samples with two drops. The requirements for best activation must be worked out for each specimen of papain. These requirements vary with the papain preparation and the extent of inactivation.

Hemoglobin tests for papain activity were carried out according to the directions of Anson (1938). Anson's procedure for the cyanide activation of papain was followed; 0.5 c.c. of papain solution was treated with five drops of 2 *M* NaCN for three minutes, and the solution was then diluted to a final concentration of 0.02 *M* cyanide. Papain not activated by alkaline cyanide was determined in the absence of all cyanide.

Extent of the digestion of beef was followed by precipitating the meat protein with trichloracetic acid and estimating the unprecipitated digestion products present in the acid filtrates, by means of the Folin-Ciocalteau (1927) phenol reagent.

Papain digest of beef was prepared by adding 100 mg. of papain to 100 grams of ground beef and heating at 70°C. for one to two hours. The papain was then inactivated in an autoclave (15 lb.) or an Arnold steamer.

Pepsin digest of beef was prepared by adding a solution of 1.25 grams of commercial pepsin (1:1,000) to 125 grams of ground beef previously brought to pH 2 with 0.5 *N* HCl. Toluene was added, and the meat was incubated at 37°C. (98.6°F.) overnight. The pH was brought back to 5.5 with NaOH, and the pepsin inactivated by heating at 80 to 100°C. (176 to 212°F.) for about half an hour. The digest was filtered through cheesecloth.

SUMMARY

Papain that is inactive by the usual tests may be very active in the digestion of meat or other products containing sulfhydryl groups or capable of producing them during proteolysis. Activation increases as digestion progresses. Preliminary activation of papain for use on such substrates is therefore unnecessary. Tests of the original activity of the enzyme give little or no indication of its potency in such cases. Peptic digests of meat also produce rapid activation of crystalline chymopapain. This may be prevented by treating the digest with enough iodoacetamide to abolish the nitroprusside test.

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RESPONSE OF RATS MAINTAINED ON DIETS FUMIGATED WITH METHYL BROMIDE

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At the present time large quantities of many types of foodstuffs are being fumigated with methyl bromide, and because of the economy and efficiency of this material, its use as a fumigant for foodstuffs is steadily increasing. With this widespread use of methyl bromide as a fumigant in the food industries, it is important to have information concerning the nature of the residues in fumigated foodstuffs and the physiological significance of such residues.

Analytical studies on total bromide content of methyl bromide-fumigated foods have been published by Dudley (1939); Dudley, Miller, Neal, and Sayers (1940); Dudley and Neal (1942); Laug (1941); Mackie (1938); Roehm, Shrader, and Stenger (1942); Roehm, Stenger, and Shrader (1943); Shrader, Beshgetoor, and Stenger (1942); and Stenger, Shrader, and Beshgetoor (1939). In general, the total bromide residue is rather low in most fresh fruits, dried fruits, fresh vegetables, and whole grains, but higher in milled grains, cheese, and nut meats. The quantity of bromide retained is likely to be less in commercial fumigation than in laboratory fumigation. Recent work by Laug (1941); Roehm, Shrader, and Stenger (1942); Roehm, Stenger, and Shrader (1943); and Shrader, Beshgetoor, and Stenger (1942) has shown conclusively that the bromide retained by a wide variety of foodstuffs following fumigation with methyl bromide exists almost entirely as inorganic bromide after standing for a few days.

Dudley and co-workers (1940, 1942) have reported feeding experiments in which rats were maintained for 20 weeks on a diet fumigated with three pounds of methyl bromide per 1,000 cubic feet and containing 24 to 30 milligrams of bromine per 100 grams, with the production of little or no deleterious effect.

In view of the importance of such experimental studies in determining the physiological significance of the bromide residue in fumigated foods, rats have been maintained in this laboratory for one year on diets fumigated with two and six pounds of methyl bromide per 1,000 cubic feet. In order to have a parallel study on the response of animals to comparable amounts of inorganic bromide, a group of rats was fed the basic diet containing 0.1 per cent of sodium bromide.

EXPERIMENTAL PROCEDURE AND RESULTS

Diet I—Control: A modified Sherman diet devised by Hawk and Oser and published by Hawk and Bergeim (1931) was used as the control diet throughout the experiment. This basic diet, designated as Diet I, contained the following constituents:

Whole wheat, ground	55%
Dried whole milk	25%
Dried meat residue	12%
Dried brewer's yeast	5%
Calcium carbonate	1%
Iodized table salt	2%

This diet was made up monthly using freshly ground whole wheat. The average total bromide content of 21 batches of this basic diet was 1.3 milligrams per 100 grams. All of the rats on each of the diets received a supplement of approximately two grams of unfumigated cabbage three times a week.

Diet I 2F: The basic diet was sacked in three-kilogram paper bags and fumigated with two pounds of methyl bromide per 1,000 cubic feet at a temperature of approximately 26.7°C.(80°F.) and a humidity of 60 to 70 per cent. Six to eight bags were fumigated at one time in a 500-cubic foot chamber. After 24 hours the bags were removed from the fumigation vault and allowed to aerate for one week. The food was then sampled for bromide analysis and fed to the experimental animals. Fresh batches were prepared and fumigated at monthly intervals. The average total bromide content of 21 batches was 26.2 milligrams per 100 grams as determined by the method of Shrader, Beshgetoor, and Stenger (1942).[1]

Studies conducted in a similar manner to those reported by Roehm, Shrader, and Stenger (1942) showed conclusively that the bromide residue in these fumigated rat diets after aeration was inorganic in nature.

Diet I 6F: This diet was prepared and handled in exactly the same way as Diet I 2F except that the control diet was fumigated with six pounds of methyl bromide per 1,000 cubic feet. The average total bromide content of the 21 batches was 63.7 milligrams per 100 grams.

Diet I + NaBr: Sodium bromide was added to the control diet to the extent of 0.1 per cent. The total bromide content of this Diet I + NaBr averaged 79.0 milligrams Br per 100 grams.

Experimental Animals: A total of 25 male rats and 32 female rats was started on each of the fumigated diets and on the control diet, while 10 males and 15 females were started on the diet containing 0.1 per cent NaBr. The rats were approximately 25 to 30 days old when placed on the experiment and were apportioned among the four groups so that they were well matched in regard to litter, sex, age, and weight.

Growth and Survival: Throughout the experimental period of one year the rats on each of the diets appeared to be in excellent condition and showed no evidence of intoxication. Semiweekly weight records, which were kept on each rat, show conclusively that the rats on the fumigated diets and on the diet containing 0.1 per cent NaBr grew as well as those on the control diet. The average body weights of all the rats in each group are given graphically (Fig. 1).

Practically all of the deaths that occurred during the course of the experiment were due to pneumonia; however, the incidence of infection was as great in the control groups as in the experimental groups. Composite weight curves, drawn solely from the body weights of the rats that

[1] The bromide analyses were carried out by Dr. S. A. Shrader and Dr. V. A. Stenger.

survived for a full year, are practically identical to those given in Fig. 1. Thus it is evident that the growth of the rats on the experimental diets was unquestionably normal.

Histopathology: At the end of the experiment the rats were killed, and tissues from representative animals in each group were prepared for microscopic study. Tissues from 61 rats were examined. Regularly, the liver, kidney, spleen, adrenal, pancreas, and heart were sectioned; although in some cases several other organs, such as muscle, lung, testis, and stomach, were also included. Microscopic examination of these sections failed to reveal any significant difference in the tissues from the rats in the control group and those from the animals on the experimental diets.

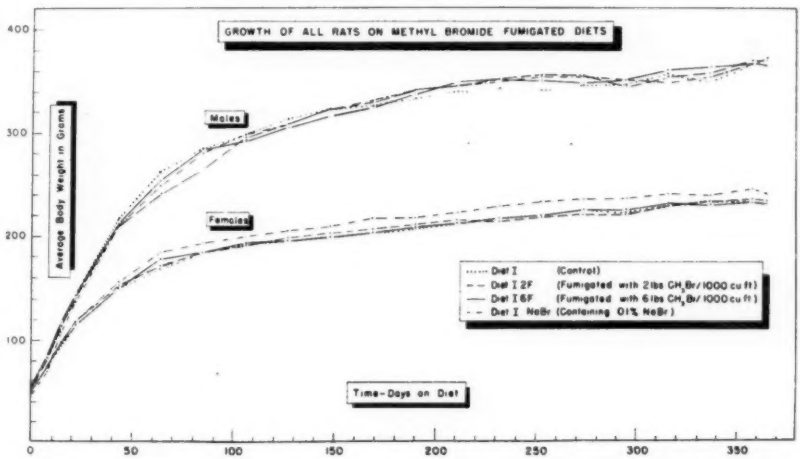

FIG. 1. The response of rats maintained on diets fumigated with methyl bromide.

Organ Weights: When the rats were autopsied at the end of the experiment, weights were obtained on the liver, kidneys, spleen, and, in the case of the males, on the testes (Fig. 2). It is evident that the values obtained in the different groups are practically identical.

Hematology: At the end of the experimental period of one year blood examinations were made upon 82 representative rats. No significant difference was found in the erythrocyte count, hemoglobin concentration, leucocyte count, differential count, or blood specific gravity between the control rats and those on the methyl bromide-fumigated diets or food containing 0.1 per cent sodium bromide.

Metabolism: At intervals during the experiment, rats from each of the groups were placed in metabolism cages so that urine specimens could be collected and an estimation of the food intake made. Two rats were placed together in one cage and a four-day composite urine sample collected. Thirteen such collections were made during the experimental period of one year; the results of this work are tabulated (Table 1).

TABLE 1

Bromide Intake and Excretion of Rats on Each Diet

Diet	Number of tests	Bromide intake (calculated from quantity of food eaten)	Urinary excretion of bromide
		mg. Br/kg./day	*mg. Br/kg./day*
Diet I (control)	13	0.7	0.7
Diet I 2F (fumigated with 2 lb. CH_3Br/1,000 cu. ft.)	13	16.4	9.8
Diet I 6F (fumigated with 6 lb. CH_3Br/1,000 cu. ft.)	13	36.4	20.7
Diet I containing 0.1% NaBr	11	40.9	24.3

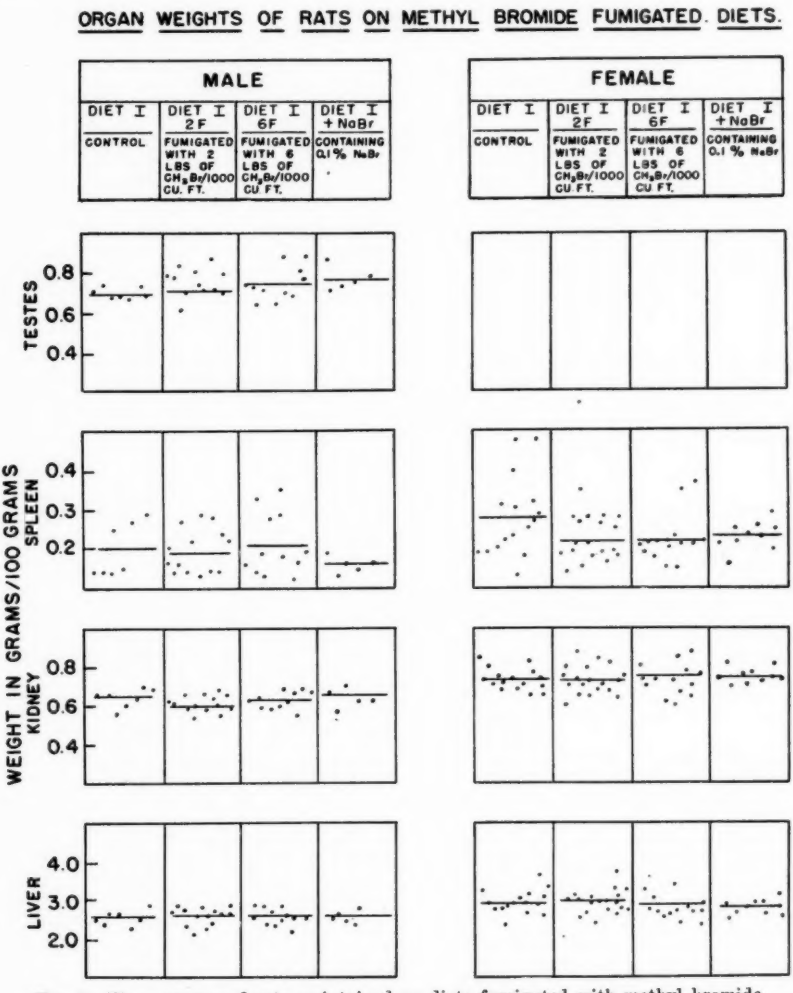

FIG. 2. The response of rats maintained on diets fumigated with methyl bromide.

Quite probably the bromide intake based upon the quantity of food eaten is somewhat high owing to the inevitable loss of some food, even in well-constructed metabolism cages. On the other hand, the values based on the urinary excretion are probably a little low owing to incomplete collection of the four-day composite and other technical difficulties. Thus, the actual bromide intake probably lies somewhere between the two values given by these calculations.

TABLE 2

Chloride and Bromide Content of Rat Tissues

TOTAL BROMIDE

Diet	Mg. Br/100 ml. blood	Mg. Br/100 gm. desiccated tissue				
		Liver	Kidney	Muscle	Skin	Brain
Diet I (control)	2.7	0.6	2.6	0.9	1.2	1.5
Diet I 2F (fumigated with 2 lb. CH_3Br/1,000 cu. ft.)	7.0	7.3	20.6	3.7	11.1	7.0
Diet I 6F (fumigated with 6 lb. CH_3Br/1,000 cu. ft.)	19.6	16.0	48.6	8.6	21.9	15.0
Diet I containing 0.1% NaBr	27.4	25.1	75.2	13.0	35.7	17.6

TOTAL CHLORIDE

Diet	Mg. Cl/100 ml. blood	Mg. Cl/100 gm. desiccated tissue				
		Liver	Kidney	Muscle	Skin	Brain
Diet I (control)	280	348	1,020	202	469	521
Diet I 2F (fumigated with 2 lb. CH_3Br/1,000 cu. ft.)	287	371	1,050	216	528	497
Diet I 6F (fumigated with 6 lb. CH_3Br/1,000 cu. ft.)	268	357	1,004	218	466	505
Diet I containing 0.1% NaBr	235	348	953	205	453	500

CHLORIDE/BROMIDE RATIO

Diet	Blood	Liver	Kidney	Muscle	Skin	Brain
Diet I (control)	104	580	393	224	391	348
Diet I 2F (fumigated with 2 lb. CH_3Br/1,000 cu. ft.)	41	51	51	58	48	71
Diet I 6F (fumigated with 6 lb. CH_3Br/1,000 cu. ft.)	14	22	21	25	21	34
Diet I containing 0.1% NaBr	9	14	13	16	13	28

Tissue Analyses: At the end of the experimental period of one year, the blood, liver, kidney, muscle, skin, and brain from about 60 representative animals were analyzed for total bromide and total chloride. Protein-free blood filtrates were prepared using tungstic acid, while the organs were minced and dried to constant weight at 100°C.(212°F.) before the quantitative determinations were made. The total halide was determined as chloride by a modification of the Carius method, used by Kimball, Wittenburg, and McFadyen (1937). The total bromide was determined by the method described by Shrader, Beshgetoor, and Stenger (1942). The results of this work are summarized (Table 2).

It is evident that even though the feeding of methyl bromide-fumigated foods or a diet containing 0.1 per cent NaBr caused an increase in the total bromide of the blood and tissues, the bromide concentration attained was quite low. The total halide remained practically constant in all the animals. The chloride/bromide ratio in each group of rats ingesting the same quantity of bromide in the food was essentially constant in the liver, kidney, muscle, and skin; however, this ratio was definitely higher in the brain than in the other tissues examined.

DISCUSSION

Since in commercial fumigation practice methyl bromide is commonly used in a concentration of one to two pounds per 1,000 cubic feet, it was advisable that one group of rats should be fed a diet fumigated at this upper limit and that another group should be fed a diet fumigated with a reasonable excess of methyl bromide; six pounds per 1,000 cubic feet was selected for this second level. The diet containing sodium bromide was designed to contain slightly more inorganic bromide than the total bromide left after aeration in the diet fumigated with six pounds methyl bromide per 1,000 cubic feet. This residual bromide in the fumigated rat diet after aeration is almost entirely inorganic in nature.

The average residual bromide of 26.2 milligrams per 100 grams found in the rat diet fumigated with two pounds methyl bromide per 1,000 cubic feet is much higher than that commonly found in fumigated fresh fruits, dried fruits, fresh vegetables, and whole grains and is of the same order as the highest values recorded for milled cereal products, cheese, and nut meats by Dudley (1939); Dudley and Neal (1942); Roehm, Shrader, and Stenger (1942); Roehm, Stenger, and Shrader (1943); and Shrader, Beshgetoor, and Stenger (1942). The average residual bromide of 63.7 milligrams per 100 grams present in the rat diet fumigated with six pounds methyl bromide per 1,000 cubic feet is well above the residual bromide content of foodstuffs that have undergone proper commercial fumigation.

It is evident from the experimental results that male and female rats, maintained for one year on diets fumigated at the levels of two and six pounds of methyl bromide per 1,000 cubic feet, as well as rats fed a diet containing 0.1 per cent NaBr, made normal weight gains and showed no evidence of intoxication or nutritional deficiency throughout the experimental period. Histopathological, hematological, and organ-weight studies on animals that had been maintained on the diets for one year failed to reveal any difference between the control and experimental groups.

This work is in agreement with that of Dudley and co-workers (1940, 1942), who conclude:

"Our animal experiments on feeding rats food containing 24 to 30 mg. of bromine per 100 gm. of food for 20 weeks caused little or no deleterious effects. Similarly, feeding experiments with rabbits for a period of 52 weeks with food containing from 5.55 to 9.36 mg. of bromine per 100 gm. of food caused little or no deleterious effect. These foods were fumigated with three pounds of CH_3Br per 1,000 cubic feet under laboratory conditions and had, therefore, a higher bromine content than found under commercial conditions. It appears, therefore, that such amounts of bromine as encountered in foods fumigated with methyl bromide, under commercial conditions, would, on the basis of our evidence, probably not contain sufficient quantities of bromine

residues to produce deleterious effects. For this reason milled cereal products fumigated with one pound of methyl bromide per 1,000 cubic feet will probably offer no risk when such food is consumed."

The quantitative information gained from the diet analyses, metabolism studies, and blood-bromide determinations are tabulated (Table 3). Even the highest blood-bromide level found in any of the rats on these fumigated diets or on the diet containing 0.1 per cent sodium bromide is far below the blood-bromide concentration commonly associated with bromism. Thus, the highest bromide concentration in the blood of any of these rats was below 30 milligrams per 100 milliliters, while a concentration of 100 to 150 milligrams per 100 milliliters is usually associated with the first stages of bromism and only rarely are any clinical symptoms

TABLE 3

Bromide Content of Diets, Bromide Ingestion, and Blood-Bromide Concentrations in Rats

Diet	Average total bromide content of diet	Average quantity of bromide ingested per day	Average total bromide concentration in the blood
	mg./100 gm.	*mg./kg./day*	*mg./100 ml.*
Diet I (control)	1.3	0.7	2.7
Diet I 2F (fumigated with 2 lb. CH_3Br/1,000 cu. ft.)	26.2	9.8-16.4	7.0
Diet I 6F (fumigated with 6 lb. CH_3Br/1,000 cu. ft.)	63.7	20.7-36.4	19.6
Diet I containing 0.1% NaBr	79.0	24.3-40.9	27.4

exhibited by patients with a lower blood bromide. In the stuporous stage of bromism the blood-bromide concentration may be 300 milligrams per 100 milliliters or greater, according to Moore, Sohler, and Alexander (1940).

Flinn (1941) has reported that in a group of individuals taking two grams of sodium bromide daily (approximately 20 milligrams bromide per kilogram per day) for four months the average blood bromide was 16.55 milligrams per 100 milliliters, while in a group receiving three grams daily (approximately 30 milligrams bromide per kilogram per day) the average bromide concentration in the blood was 50.09 milligrams per 100 milliliters. In none of these individuals receiving two to three grams of sodium bromide daily (approximately 20 to 30 milligrams bromide per kilogram per day) over a period of four months was there any indication of deleterious effects as determined by careful periodic physical examinations in which special attention was given to the following factors: nervous reactions; reaction time to light signals; pulse rate; blood pressure; electrocardiogram; oxygen capacity; carbon dioxide capacity; hemoglobin; and changes in the white and red cell count.

In view of the results of our experimental work, as well as that of others, it is evident that foodstuffs fumigated with the usual commercial concentrations of one to two pounds of methyl bromide per 1,000 cubic feet and properly aerated will contain no residues which might have a

deleterious effect, even when such fumigated foods are included as a large part of the human dietary.

CONCLUSIONS

Rats maintained for one year on diets fumigated with two and six pounds of methyl bromide per 1,000 cubic feet and having an average bromide content of 26.2 and 63.7 milligrams per 100 grams, respectively, made normal weight gains. These rats showed no evidence of intoxication or nutritional deficiency as determined by hematological, histopathological, analytical, and organ-weight studies. Similar results were obtained with rats fed a diet containing 0.1 per cent sodium bromide.

Since it has been shown that rats can be maintained for one year on diets fumigated with two and six pounds of methyl bromide per 1,000 cubic feet with the production of no discernible ill effects, it is concluded that foodstuffs, fumigated with the usual commercial concentrations of one to two pounds of methyl bromide per 1,000 cubic feet and properly aerated, will contain no residues which might have a deleterious effect, even when such fumigated foods are included as a large part of the human dietary.

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ASCORBIC ACID CONTENT OF 39 VARIETIES OF SNAP BEANS

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In the program of breeding for high-quality snap beans at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., it has been necessary to analyze a number of varieties for their ascorbic acid (vitamin C) content as a basis for incorporating the additional factor of high vitamin C content in the newly developed varieties.

This report is concerned with the ascorbic acid content of 39 commercial varieties of snap beans.

EXPERIMENTAL PROCEDURE

Twenty varieties of snap beans of the pole type and 19 varieties of the bush type were grown in rows 32 feet long in randomized blocks in two series in the spring of 1942 and again in the fall of the same year. A few other varieties were analyzed during one season only and will be mentioned later. Two or more pickings of pods were taken from each series for ascorbic acid determination, making a minimum of four determinations on each variety for each season. Leaves for analysis were also gathered from 37 of the varieties. In the spring, the leaf samples were taken at the same time as the first picking of pods. In the fall one picking of leaves was made when the plants were flowering. Another picking of the pole bean leaves was made at the time of the second picking of pods. The leaf samples were taken from the topmost, nearly full-sized leaves on the plant. These leaves were presumably among those physiologically most active from the standpoint of synthesizing power. All samples of leaves were analyzed within an hour after harvest and all pods were analyzed within an hour or placed in storage at 2.2°C.(36°F.), with 90 to 100 per cent relative humidity, and analyzed within 24 hours. Statistical constants were computed from variance analyses of the sets of data.

A modification of the Morell (1941) method was used for the determination of ascorbic acid. A sample consisting of 25 to 40 grams of bean pods or 10 to 15 grams of leaves was extracted with 200 ml. of one-per cent metaphosphoric acid for two minutes on a Waring blender at high speed, then filtered through fluted Whatman No. 12 filter paper. After discarding the first portion of the filtrate a one-ml. aliquot was placed into a colorimeter test tube containing 10 ml. of a solution of 2,6-dichlorophenol-indophenol (concentration, 17 mg. per liter) and read in an Evelyn photoelectric colorimeter within 30 seconds. Chemical blanks and their center settings were obtained by using one ml. of one-per cent metaphosphoric acid in 10 ml. of the dye solution. Center settings for the samples were obtained by using one ml. of the sample filtrate and decolorizing the remaining dye with a few crystals of ascorbic acid.

The ascorbic acid content of the sample was calculated by using an equation derived by the method of least squares from readings on aliquots of pure ascorbic acid solution of various concentrations. The dilution effect of the moisture in the fresh sample was considered in the calculation.

Preliminary experiments showed that the three-per cent metaphosphoric acid used by Morell can be replaced by a one-per cent solution

TABLE 1

Comparison of Several Modifications of Photometric Ascorbic Acid Methods

Sample		Modified Morell method	Heinze-Kanapaux method	Loeffler-Ponting method	Standard deviation	Required for significant difference at 5-per cent level
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*		
Snap beans	1	32.5	32.5	32.5	.....	.....
	2	32.0	32.2	32.3	.....	.....
	3	29.2	29.6	29.7	.....	.....
	4	31.1	31.1	30.9	.....	.....
	5	31.2	31.1	31.0	.....	.....
	6	26.2	26.2	25.5	.....	.....
	7	30.6	30.8	30.5	.....	.....
	8	32.5	31.7	32.0	.....	.....
	9	35.8	34.5	34.8	.....	.....
	10	32.4	31.7	32.2	.....	.....
Average		31.35	31.14	31.14	.318	.30
Snap beans	11	18.0	18.0	.....	.....	.....
	12	20.8	21.3	.....	.....	.....
	13	20.8	20.9	.....	.....	.....
	14	22.7	22.4	.....	.....	.....
Average		20.58	20.65	.....	.238	.54
Potato	1	4.9	4.9	.....	.....	.....
	2	6.9	6.8	.....	.....	.....
	3	5.8	5.8	.....	.....	.....
	4	6.5	6.2	.....	.....	.....
Average		6.03	5.93	.....	.100	.22
Tomato	1	14.4	14.9	.....	.....	.....
	2	13.0	13.3	.....	.....	.....
	3	13.5	13.8	.....	.....	.....
	4	21.3	20.7	.....	.....	.....
Average		15.55	15.68	.....	.349	.78

without loss of accuracy. That the buffers can also be omitted is shown (Table 1) where no significant differences are noted between the modified Morell method using buffers and the Heinze-Kanapaux method in which no buffers are used. Loeffler and Ponting (1942) have adopted a procedure eliminating the use of buffers. They extract with one-per cent metaphosphoric acid for five minutes in a Waring blender with a larger quantity of acid and use a smaller volume of a more dilute dye. Numerous runs

with snap beans comparing the use of 350 ml. of acid and five-minute extraction with 200 ml. of acid and two-minute extraction gave slightly but not significantly higher values for the two-minute extraction.

For the data (Table 1) the samples were ground with 200 ml. of acid for two minutes and aliquots of the same extract were carried through the different procedures.

RESULTS

In the spring the average ascorbic acid content (Tables 2 and 3) ranged from 23 to 33.2 mg. per 100 grams of fresh weight for the pods

TABLE 2

Ascorbic Acid Content and Rank of 19 Varieties of Bush Snap Bean Pods of Spring and Fall Crops of 1942

Variety	Spring 1942		Fall 1942		Mean for spring and fall	Rank
	Mean of 2 pickings from 2 series	Rank	Mean of 4 pickings from 2 series	Rank		
	mg./ 100 gm.		*mg./ 100 gm.*		*mg./ 100 gm.*	
Unrivalled Wax	31.9	2	33.1	1	32.5	1
Low's Champion	33.2	1	27.6	4	30.4	2
Giant Stringless	31.5	3	27.5	5	29.5	3
Masterpiece	30.6	4	28.3	2	29.5	3
Bountiful	29.0	7	27.9	3	28.5	5
Sure Crop Wax	30.5	5	25.2	7	27.8	6
Dwarf Horticultural	28.3	9	25.6	6	26.9	7
Tennessee Green Pod	30.2	6	23.2	11	26.7	8
Tendergreen	28.2	10	25.0	9	26.6	9
Brittle Wax	28.9	8	24.0	10	26.5	10
Stringless Red Valentine	26.2	13	25.2	7	25.7	11
Stringless Black Valentine	27.3	11	22.2	12	24.7	12
Burpee Stringless Green Pod	26.3	12	19.9	14	23.1	13
Early Refugee Stringless	24.8	15	21.3	13	23.0	14
U. S. No. 5 Refugee	25.8	14	19.6	15	22.7	15
Idaho Refugee	24.5	16	18.7	18	21.6	16
U. S. No. 1 Refugee	23.3	18	19.2	16	21.2	17
Wisconsin Refugee	23.0	19	19.1	17	21.0	18
Stringless Refugee	23.4	17	16.2	19	19.8	19
Average	27.7	....	23.6	....	25.7	....
Significant difference 5%	4.79		4.58		2.55	
Significant difference 1%	6.38		6.28		3.38	

of the bush beans and from 21.6 to 38.2 mg. for the pole beans. In the fall the averages ranged from 16.2 to 33.1 mg. for the bush beans and from 17.9 to 31.2 mg. for the pole beans. Mammoth Horticultural and Alabama 3 were consistently the two highest ranking varieties among the pole beans. Unrivalled Wax, Low's Champion, Giant Stringless, and Masterpiece were within the highest five among the bush varieties in both seasons. Bountiful ranked seventh in the spring and third in the fall, with a rank of five for the average of both seasons.

Several varieties not included in the spring data but analyzed in the fall were Cooper Wax with 23.1 mg.; Tennessee Wonder, 21.6 mg.; Pencil

Pod Black Wax, 21.3 mg.; Large White Marrow, 19.6 mg.; and Full Measure, 18.9 mg. The Urd bean (*Phaseolus mungo* L. Mant.) contained 13.8 mg. which was much lower than any of the other beans tested.

One variety of the pole type, Missouri Wonder, was analyzed in the spring and was not repeated in the fall data. It contained 19.3 mg. and ranked lower than any other pole variety.

Tables 4 and 5 give the ascorbic acid content of the leaves of some of the same varieties listed in Tables 2 and 3. The correlation coefficient for

TABLE 3

Ascorbic Acid Content and Rank of 20 Varieties of Pole Snap Bean Pods of Spring and Fall Crops of 1942

Variety	Spring 1942		Fall 1942		Mean for spring and fall	Rank
	Mean of 2 pickings from 2 series	Rank	Mean of 3 pickings from 2 series	Rank		
	mg./ 100 gm.		*mg./ 100 gm.*		*mg./ 100 gm.*	
Mammoth Horticultural	38.2	1	31.2	1	34.7	1
Alabama 3	35.4	2	25.4	2	30.7	2
Alabama 4	30.2	3	22.4	9	26.3	3
Early Blue Lake	26.2	8	24.8	3	25.5	4
Stringless Blue Lake 4161	27.5	5	22.2	11	24.9	5
Alabama 2	25.1	10	23.4	6	24.3	6
Stringless Blue Lake 4057	24.2	14	23.8	4	24.0	7
Striped Creaseback	24.5	12	23.6	5	24.0	7
Kentucky Wonder Wax	25.0	11	22.3	10	23.6	9
Ideal Market	24.4	13	22.2	12	23.4	10
White Kentucky Wonder	27.2	6	18.6	18	22.9	11
Stringless Blue Lake 4157	27.6	4	18.1	19	22.9	11
Stringless Blue Lake 4060	22.0	19	22.9	8	22.5	13
Stringless Ideal Market 4176	25.7	9	19.1	17	22.4	14
Rust Resistant Kentucky Wonder	21.6	20	23.0	7	22.3	15
Alabama 1	26.4	7	17.9	20	22.2	16
Kentucky Wonder	23.2	15	19.3	15	21.3	17
Stringless Blue Lake 4160	23.0	16	19.3	15	21.2	18
Stringless Ideal Market 4179	22.5	18	19.8	13	21.2	18
Late Blue Lake	22.8	17	19.5	14	21.1	20
Average	26.1	....	21.9	....	24.1	....
Significant difference 5%	4.74		5.15		2.82	
Significant difference 1%	6.30		7.04		3.73	

ascorbic acid content of leaves and pods of bush beans picked on the same day in the spring was 0.426.[1] For the second picking of pods approximately one week later the correlation coefficient was 0.161; for an average of the two pickings (means given in Tables 2 and 4) the coefficient was —0.02. For the pole bean pods picked on the same day as the leaves the correlation coefficient was —0.211, and for a second picking of pods about nine days later the value was —0.195. The means (Tables 3 and 5) give a coefficient of —0.394 which is not significant.

In the fall, when the leaves were gathered at the time the plants were flowering and the pods harvested three to six weeks later, the correlation

coefficients for the four pickings of bush beans were 0.183, 0.339,[1] 0.317,[1] and 0.281. The means (Tables 2 and 4) gave a value of 0.372. For pole beans with leaves gathered at the same time the correlation coefficients were 0.574,[1] 0.365,[1] and 0.095, respectively, for the three pickings. The means (Tables 3 and 5) for the pole beans gave a value of 0.176. Leaves from the pole bean varieties were also gathered on October 13, at the time of the first picking of pods. The correlation coefficients for this picking and the two succeeding pickings of pods were 0.345, 0.180, and 0.032,

TABLE 4

Ascorbic Acid Content and Rank of 19 Varieties of Bush Snap Bean Leaves of Spring and Fall Crops of 1942

Variety	Spring 1942		Fall 1942		Mean for spring and fall	Rank
	Mean of 1 picking from 2 series	Rank	Mean of 1 picking from 2 series	Rank		
	mg./ 100 gm.		*mg./ 100 gm.*		*mg./ 100 gm.*	
Tennessee Green Pod	167.8	12	142.2	1	155.0	1
Masterpiece	200.3	1	103.4	15	151.8	2
U. S. No. 5 Refugee	190.2	3	110.5	8	150.4	3
Idaho Refugee	192.2	2	102.2	16	147.2	4
U. S. No. 1 Refugee	185.7	5	108.3	11	147.0	5
Low's Champion	162.1	13	131.3	2	146.7	6
Sure Crop Wax	184.7	6	108.4	10	146.6	7
Tendergreen	188.6	4	104.6	14	146.6	7
Giant Stringless	171.2	8	112.4	6	141.8	9
Stringless Refugee	177.0	7	106.2	13	141.6	10
Bountiful	168.2	11	111.7	7	139.9	11
Early Refugee Stringless	169.5	9	110.2	9	139.9	11
Unrivalled Wax	149.9	16	123.5	3	136.7	13
Burpee Stringless Green Pod	158.2	15	113.8	5	135.9	14
Brittle Wax	161.0	14	102.0	17	131.5	15
Stringless Black Valentine	169.2	10	88.5	18	128.8	16
Stringless Red Valentine	141.8	17	106.9	12	124.4	17
Dwarf Horticultural	119.8	19	115.0	4	117.4	18
Wisconsin Refugee	137.9	18	79.7	19	108.8	19
Average	168.2	...	109.5	....	138.8	....
Significant difference 5%	37.5		16.2		19.86	
Significant difference 1%	50.9		22.0		26.56	

none of which is significant. All correlation analyses were made on data expressed in the nearest milligram.

DISCUSSION

Pods from the spring harvest of both the pole and bush varieties were significantly higher than those from the fall harvest in ascorbic acid content. This agrees with the findings of Wade and Kanapaux (1943) for the ascorbic acid content of a number of genetic strains of snap beans but differs from that of Mack, Tapley, and King (1939), who report little

[1] Significant at the five-per cent level.

variation in Refugee over a period of three years. However, their plants were grown during only one season of each year. Some individual varieties among those analyzed in the present study have been found to be very consistent in their ascorbic acid content from season to season and from year to year. The average values for Cooper Wax were 23.1 for 16 determinations during the spring of 1941, 20.6 for 16 determinations during the fall of 1941, 23.1 for one determination in the spring of 1942, and 23.1 for eight determinations during the fall of 1942.

TABLE 5

Ascorbic Acid Content and Rank of 18 Varieties of Pole Snap Bean Leaves of Spring and Fall Crops of 1942

Variety	Spring 1942		Fall 1942		Mean for spring and fall	Rank
	Mean of 1 picking from 2 series	Rank	Mean of 1 picking from 2 series	Rank		
	mg./ 100 gm.		*mg./ 100 gm.*		*mg./ 100 gm.*	
Late Blue Lake	158.3	1	122.7	6	140.5	1
Kentucky Wonder Wax	151.3	3	128.9	4	140.1	2
Stringless Ideal Market 4176	151.3	2	127.3	5	139.3	3
Alabama 2	137.0	7	141.0	1	139.0	4
Striped Creaseback	148.1	4	113.3	11	130.7	5
Kentucky Wonder	144.9	6	116.1	8	130.5	6
Mammoth Horticultural	123.4	12	134.6	2	129.0	7
Rust Resistant Kentucky Wonder	134.5	9	118.7	7	126.6	8
Stringless Blue Lake 4057	136.1	8	115.4	9	125.8	9
White Kentucky Wonder	118.4	14	132.0	3	125.2	10
Stringless Ideal Market 4197	146.3	5	100.5	14	123.4	11
Alabama 3	118.9	13	114.0	10	116.5	12
Early Blue Lake	128.4	10	95.8	15	112.1	13
Stringless Blue Lake 4160	114.2	17	109.5	12	111.9	14
Stringless Blue Lake 4161	114.7	16	106.4	13	110.6	15
Ideal Market	135.3	11	68.8	18	102.1	16
Stringless Blue Lake 4060	114.8	15	82.1	16	98.4	17
Stringless Blue Lake 4157	106.7	18	79.7	17	93.2	18
Average	132.4	....	111.5	....	121.9	....
Significant difference 5%	48.0		29.5		27.74	
Significant difference 1%	65.4		40.4		37.05	

With only one exception, Tennessee Green Pod, the same 10 bush varieties that had the highest ascorbic acid content in the pods in the spring were again among the highest 10 in the fall. The agreement between the spring and fall values for the pole varieties was not so consistent. Here only five of the first 10 varieties in the spring test were again among the highest 10 in the fall. However, this indicates that the varieties do tend to range in the same order for ascorbic acid content under different environmental conditions. Murphy (1942) has found this to be true in cabbage and tomatoes. She states: "A variety nutritionally superior by virtue of its genes is proportionately superior even when subjected to an unfavorable environment."

Mayfield and Richardson (1939) report values of 0.27 and 0.19 mg. of ascorbic acid per gram for Burpee Stringless Green Pod for two different years. Our values for spring and fall harvest were 26.3 and 19.9 mg. per 100 gm., respectively, for the same variety.

Mack, Tapley, and King (1939), in their study of different varieties of snap beans, found the vitamin C content of the entire pods as normally harvested to range in the following order: Tendergreen 0.24, Ideal Market 0.20, Kentucky Wonder Wax 0.19, Refugee 0.19, and Kentucky Wonder 0.18 mg. per gm. The values reported in this paper for the same varieties were Tendergreen 26.6, Kentucky Wonder Wax 23.6, Ideal Market 23.4, Refugee 22.7, and Kentucky Wonder 21.3 mg. per 100 gm. The varieties are very nearly in the same order with reference to one another although the values given here are slightly higher than those of Mack, Tapley, and King.

Wade and Kanapaux (1943) reported on three commercial varieties included in their 49 strains in 1941. Bountiful gave readings of 28.7 and 26.3 mg. per 100 gm. for spring and fall harvests, respectively, in 1941, and 29 and 27.9 mg. for spring and fall of 1942. Stringless Black Valentine gave readings of 25.8 and 25.4 mg. for spring and fall of 1941, and in 1942 the values were 27.3 and 22.2 mg. Brittle Wax gave readings of 25.6 and 21.3 mg. in 1941, and in 1942 the values were 28.9 and 24 mg. Bountiful was higher than the other two varieties in vitamin C in all analyses but significantly so only in 1941.

When harvested at the same time or within a week or two of each other the correlation between the ascorbic acid content of the leaves of the bean plants and the pods has been erratic. In some instances the correlation coefficient was positive and in others negative. In only one case in this investigation was there a significant value at the five-per cent level. However, when the leaves were analyzed at the time the plants were flowering and the pods gathered from three to six weeks later, a significant positive correlation was found in four of seven different pickings. This would indicate that the level of vitamin C content in the plant during the earlier portion of its growing period has a closer relation to the vitamin C in the pods than the content slightly before or at the time of harvest.

SUMMARY

A more rapid method of determining the vitamin C content of snap beans, which eliminates the use of buffers, has been presented.

The vitamin C content of 39 varieties of snap beans was determined. The results indicate that varieties tend to remain in the same relative order in regard to vitamin C content in the spring and the fall harvests. The uniformity was more pronounced in the bush type than in the pole type varieties.

Little or no correlation between vitamin C content of the leaves and pods has been found when they were harvested at the same time or within two weeks of each other. A slight significant positive correlation between the vitamin C content of the leaves at the flowering stage of the plant and the pods was noted in over 50 per cent of the pickings.

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EFFECT OF TIME AND TEMPERATURE OF STORAGE ON VITAMIN C RETENTION IN CANNED CITRUS JUICES

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The pioneering work to determine the vitamin C potency of canned orange juice was done in 1931 under a private research grant to Fellers and Isham (1932) at Massachusetts State College. Excellent protection against scurvy for guinea pigs was obtained. Shortly thereafter, chemical methods for vitamin C assay were developed, and an extensive literature on vitamin C in foodstuffs has accumulated. Investigations of value to the canning operations have been made in this laboratory and some data of general interest have been obtained. In 1936 preliminary work was done, with the use of aqueous iodine, in the estimation of the vitamin C content of fresh citrus juices. During the spring of 1938 a program was initiated to collect vitamin C data along with other chemical control data routinely kept of all fruit used at the canning plant, Ross (1941). The effect of processing was studied during several seasons, and sample cans were set aside from time to time for storage experiments. From the point of view of the ultimate consumer, it was considered desirable to extend the investigations to include juice stored in open containers, simulating practices normally used in the home.

Samples of juice were drawn at intervals during the regular canning process. Freshly reamed juice was taken from 160-gallon tanks and sample cans taken after this *same* juice had been pasteurized at 87.8°C. (190°F.) and sealed into cans. In order to study the effect of storage temperature on the retention of vitamin C in canned citrus juices, a series of three experimental packs were run. The juice in each of these was from the same tank of freshly reamed orange juice. One case of 24 cans was placed in an incubator at a constant temperature of 37°C.(98.6°F.); another was placed on the bottom shelf of an electrical refrigerator where the temperature remained between 7 and 11°C.(44.6 and 51.8°F.). The third case was maintained at room temperature.

METHOD OF ANALYSIS

Early determinations were made according to the method of Bessey and King (1933). However, it was soon found that the extraction of the centrifuged solids with hot eight per cent acetic acid was not necessary, and direct titration of the untreated juice with 0.05 per cent aqueous sodium 2,6-dichlorobenzenoneindophenol (hereafter called "dye") was employed. The first pink coloration which lasted a matter of five seconds was found to be a satisfactory end point. According to Floyd and Fraps (1942) treatment of fresh or canned grapefruit juice with H_2S did not cause any increase in titratable ascorbic acid (vitamin C).

Standardization of the dye solution was first made against fresh grapefruit juice, the vitamin C of which had been determined with standard

iodine solution. However, a very satisfactory method was found by titrating a weighed quantity of dry ascorbic acid directly with the dye solution. A small amount (about 0.1 gm.) of citric acid was added to the flask containing the dry ascorbic acid previous to titration. The variability of this standardization was less than one per cent. Since the amount of dye was weighed accurately, the factor for every batch could be expected to be quite constant. This served as a double check on standardizations. Duplicate determinations of vitamin C in the juice invariably checked to less than one per cent.

Titration of canned citrus juice with 0.01 N iodine gave irregular values over a period of months in storage. An experiment wherein one per cent citric acid in water was hermetically sealed in cans and stored indicated clearly the difference in reducing action of the dissolved metals on the two testing solutions. The titration values in these cans steadily increased with time for the iodine solution but remained at zero for the dye solution.

RESULTS

Early experiments (Table 1) studying the retention of vitamin C potency in canned citrus juices showed that from 98 to 92 per cent of the vitamin C of the fresh juice remains after canning, using the patented pasteurization process employed in this canning plant. After 12 months of storage at room temperature the vitamin C content was found to range from 70 to 80 per cent of that of the fresh juice. There is a gradual loss of about 0.01 mg. per ml. per month. The data indicate a somewhat greater retention of vitamin C in canned grapefruit juice than in orange juice. However, the time of half loss is reached at the same time for both, i.e., the 22nd month of storage.

It became evident as data accumulated from numerous other experimental packs, not herein reported, that other important factors besides storage time were active in determining vitamin C retention. One such factor is temperature, which the results (Table 2) readily show.

The excellent retention of vitamin C in canned orange juice kept under refrigeration (bottom shelf of home electrical refrigerator) is evident from the data (Table 2). After one year's storage the vitamin C potency is 94 per cent of what it was just after canning and 89 per cent of the amount in the original freshly reamed juice. After one year at room temperature,[1] there is 75 per cent of the vitamin C when canned. Thus, the effect of an average 15-degree rise has been to lower vitamin C from 94 to 75 per cent. Interpolating to a cool warehouse temperature of 17°C. (62.8°F.), the retention of vitamin C could be expected to be very good, about 85 per cent after one year. The next 13-degree rise affects vitamin C content by the amount of the difference between 75 and 17 per cent. Consequently, the average effect of temperature between 24 and 37°C. (75.2 and 98.6°F.) is much greater per degree than between 9 and 24°C.(48.2 and 75.2°F.).

[1] In a 12-month period this amounts to the difference between 0.460 and 0.367 mg. per ml., or 0.042 ounce for a case of 24 No. 2 (18 oz.) cans. At the current price (Hoffman-La Roche Co.) of $1.20 per ounce for synthetic ascorbic acid, refrigeration over one year would save an amount of vitamin C worth five cents per case.

TABLE 1

Effect of Canning and Storage on Vitamin C of Canned Citrus Juices

Storage period	Vitamin C content															
	Sweetened orange juice								Grapefruit juice							
									Sweetened				Unsweetened			
	Canned[1] 5/38		Canned 4/38		Canned 4/39		Canned 4/41		Canned 5/38		Canned 4/38		Canned 5/41		Canned 3/41	
	mg./ml.	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*	*mg./ml.*	*pct.*
Fresh	.38	100	.38	100	.56	100	.59	100	.42	100	.37	100	.41	100	.50	100
Canned	.35	92	.36	95	.52	93	....	....	.40	95	.34	92	.40	98	....	....
mo.																
1	.33	87	.33	87	....	....	.53	90	.41	98	.31	84	.39	95	....	....
2	.32	84	.31	82	....	....	....	....	....	....	....	....	....	....	.49	98
3	....	....	.29	76	....	....	.48	81	.38	91	....	....	....	....	....	....
4	.31	82	....	....	....	....	....	....	....	....	....	....	....	....	.44	88
5	....	....	....	....	....	....	....	....	.35	83	.29	78	.35	85	....	....
6	....	....	....	....	....	....	.43	73	....	....	....	....	....	....	....	....
7	.30	79	....	....	....	....	....	....	....	....	....	....	.34	83	.40	80
8	....	....	.28	74	....	....	.42	71	....	....	....	....	....	....	....	....
9	.27	71	....	....	....	....	....	....	.36	86	....	....	....	....	.39	78
10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
11	....	....	....	....	....	....	.40	68	....	....	....	....	....	....	....	....
12	.27	71	.26	69	....	....	....	....	.33	79	....	....	.33	80	.37	74
21	.21	55	....	....	....	....	....	....	....	....	....	....	....	....	....	....
22	....	....	.19	50	....	....	....	....	....	....	.19	51	....	....	....	....
26	....	....	....	....	.22	39	....	....	....	....	....	....	....	....	....	....
38	.11	29	....	....	....	....	....	....	....	....	....	....	....	....	....	....
39	....	....	....	....	....	....	....	....	....	....	.10	27	....	....	....	....

[1] Enamel-lined cans and lids; all others plain tin.

Flavor in canned orange juice was found to be significantly well preserved by refrigerated storage. After a period of one year at an average temperature of 9.1°C., the juice was judged to possess a distinct orange flavor, free from caramelization, oxidation, or terpene. Taste tests were made each month by two of us in the laboratory; after the first month the juice could always be distinctly divided in order of decreasing taste appeal as that kept under refrigeration, that kept at room temperature, and that kept in the incubator. The juice stored in the refrigerator for one year was judged to possess an excellent flavor, practically equal to that of newly canned orange juice. The juice stored at room temperature for one year possessed a good flavor, having a slightly oxidized taste,

TABLE 2

Temperature-Storage Effect on Vitamin C of Canned Orange Juice[1]

Storage period	Storage in								
	Incubator			Refrigerator			Room		
	Temp.	Vitamin C		Temp.	Vitamin C		Temp.	Vitamin C	
	°C.	mg./ml.	pct.	°C.	mg./ml.	pct.	°C.	mg./ml.	pct.
Fresh	....	.519	106.0	....	.519	106.0	....	.519	106.0
Canned	....	.490	100.0	....	.490	100.0	....	.490	100.0
mo.									
1	37	.440	89.8	8	.489	99.8	21	.476	97.1
2	37	.400	81.6	10	.483	98.6	28	.469	95.7
3	37	.327	66.7	11	.479	97.7	27	.454	92.6
4	37	.265	54.1	10	.475	96.9	29	.443	90.4
5	37	.221	45.1	10	.472	96.4	28	.423	86.3
6	37	.151	30.8	10	.466	95.1	31	.404	82.4
7	37	.191	39.0	9	.476	97.1	23	.400	81.6
8	37	.137	28.0	9	.469	95.7	21	.393	80.2
9	37	.150	30.6	8	.475	96.9	15	.389	79.4
10	37	.097	19.8	9	.473	96.5	23	.383	78.2
11	37	.067	13.7	7	.469	95.7	19	.373	76.1
12	37	.081	16.5	8	.460	93.8	25	.367	74.9
Average of temperatures									
	37			9.1			24.2		

[1] Each value is the average from two separate cans; all duplicate values checked within two per cent, except for cans stored more than three months in the incubator, in which cases variations as high as 15 per cent were encountered between duplicates.

although not objectionable. After four months' storage at 37°C. the juice was definitely off-color owing to browning, and was off-flavor owing to a caramelized and oxidized flavor. After six months this juice was distinctly disagreeable and dark brown in color.

Although retention of flavor and vitamin C roughly followed each other at any one temperature, it was not possible to fix an arbitrary vitamin C potency with any one flavor.

It will be noted from the data (Table 2) that after six months' storage there is no longer a smooth, regular drop of vitamin C values. This irregularity is especially prominent for the incubated juice. Since all three packs were originally identical, the variables seem limited to two factors which could contribute to this irregularity. One such variable might be the amount of dissolved metallic ions which had accumulated in

an individual can. It is possible that stannous ions facilitate the natural reducing power of the juice, thereby adding to the protection of the vitamin C against oxidation. Headspace of air in the can is another variable between individual cans. This factor may operate alone or in combination with that of the metallic ions. Moore, Esselen, and Fellers (1943) have shown that degree of browning is associated with headspace, and that darkened samples resulting from storage at 48.9°C.(120°F.) contained no vitamin C.

Stability in Open Containers: In view of the excellent retention of vitamin C potency in canned citrus juices, the question which presents itself to the consumer concerns the potency after the can is opened. Not all of the juice may be consumed immediately, but some may be stored in the refrigerator for a day or two. The results (Table 3) show the signal stability of the vitamin C as it naturally occurs in citrus.

TABLE 3

Stability of Vitamin C in Citrus Juices in Open Containers[1]

Explanation	Storage		Vitamin C	
	Temp.	Time[2]		
	°C.	hr.	mg./ml.	pct.
Orange juice; can opened 1 month after sealing	....	0	.533	100.0
	7	16	.526	98.6
Orange juice; can opened after 5 months	....	0	.554	100.0
	8	21	.540	97.5
	8	40	.535	96.6
	8	48	.528	95.2
Orange juice; can opened after 1 year	....	0	.372	100.0
	20	3	.367	98.7
	20	19	.347	93.3
Grapefruit juice; can opened after 5 months	....	0	.397	100.0
	8	21	.388	97.8
Orange juice; fresh machine reamed; 7 hr. at 25°C.	....	0	.458	100.0
	8	16	.449	98.0
	25	23	.443	96.8
Orange juice; fresh hand squeezed: 1 hr. at 25°C. just after extraction	....	0	.574	100.0
	25	1	.574	100.0
	8	22	.570	99.4
	8	43	.562	98.0
	8	49	.562	98.0

[1] Glass bottles with plastic screw caps, and flasks covered by watch glasses. [2] Interval after can was opened or juice extracted.

DISCUSSION

When time in months is plotted against vitamin C content (Fig. 1), as given in Table 2, the much increased reaction rate (destruction) of the vitamin for the increased storage temperature is quite striking. This also can be clearly portrayed by the following analysis, using the average rate of reaction for the first six-month period. During this period the data are regular, approximating a direct proportion between vitamin C concentration and storage time. It is assumed for the purposes of the following analysis that variables other than time and temperature are not operative.

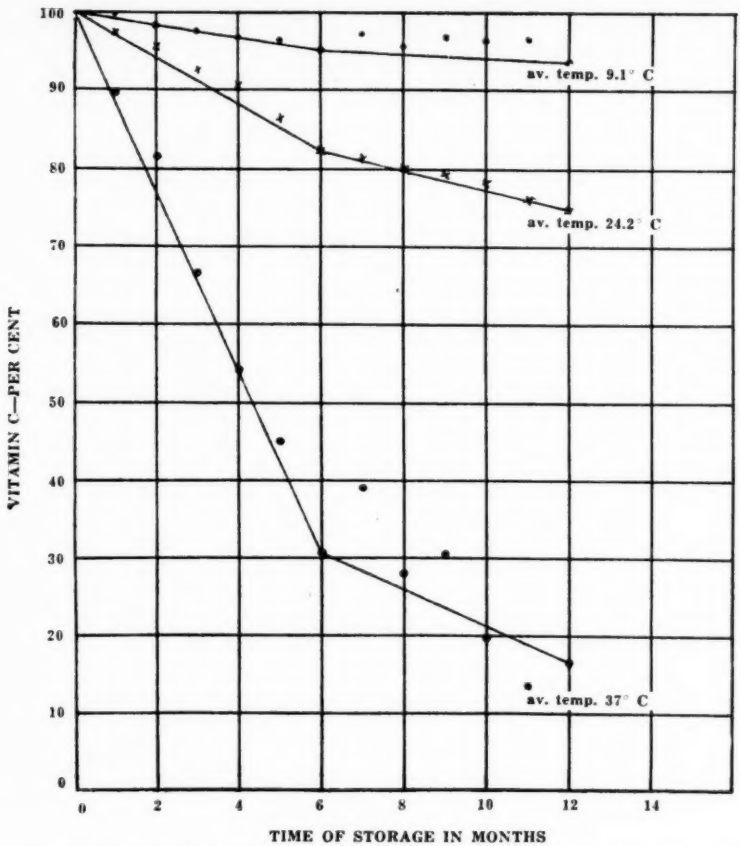

FIG. 1. Vitamin C retention in canned orange juice under different storage temperatures.

It is known empirically that a rise of 10 degrees in temperature increases the rate of most chemical and biological reactions from two to four times. The rate of reaction of vitamin C at 10°C.(50°F) was found to be 0.004 mg. per ml. per month (average from first six-month period). On that basis a table can be made as follows, doubling the reaction rate for each 10-degree rise and fitting in the values found experimentally:

10°C.—0.004 (found)	
20°C.—0.008 (calc.)	17°C.—0.0065 (calc.)
30°C.—0.016 (calc.)	27°C.—0.0130 (calc.)—0.0143(found)
40°C.—0.032 (calc.)	37°C.—0.026 (calc.)—0.0565 (found).

Thus, it has been found experimentally that between 10 and 27°C. (50 and 80.6°F.) storage temperature the rate of loss of vitamin C in canned orange juice doubles for each 10-degree rise. On the other hand, increasing the temperature from 27 to 37°C. nearly quadruples the rate

at which the reaction takes place. At 17°C. (62.6°F.) the loss of vitamin C owing to storage would be one-half as much as at 27°C.

SUMMARY

Chemical determinations of vitamin C were made on fresh and canned orange and grapefruit juices. The vitamin C potency after processing ranged from 98 to 92 per cent of that of the freshly reamed juice. Storage temperature was found to be very important to vitamin C retention in the canned juices. Very good retention is assured at 17°C., while under refrigeration 90 per cent of the vitamin C of the freshly reamed juice was retained after one year. Temperature effect was found to be disproportionately high at the higher temperatures. From 27 to 37°C. the rate of reaction of the vitamin C quadrupled, while between 10 and 27°C. the rate only doubled for a 10-degree rise in temperature. Flavor was judged to be dependent on both time and temperature of storage, roughly paralleling vitamin C retention.

The vitamin C in citrus juices in open containers is very stable. More than 95 per cent of the original (open can or freshly extracted juice) vitamin C was retained after 48 hours' storage in the refrigerator.

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A BACTERIOLOGICAL SURVEY OF SARDINE CANNING IN MAINE

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Over a period of years a considerable volume of information on the bacteriological aspects of many phases of the canning industry has been accumulated. Although this included a great deal of specialized information on sardine canning, no detailed bacteriological survey comparable to those which had been made for a number of other products had ever been done in any of the Maine sardine canning plants. The work which is here reported was prompted by the lack of full bacteriological knowledge of this important branch of the canning industry.

Preliminary work during a substantial part of one canning season served as a guide to the later work upon which this report is based. The data were obtained in a field laboratory set up at a single location and are from material collected chiefly at two factories. A limited number of tests on material from other factories, the results from the preliminary work of the preceding season at other factories, and the similarity of packing procedure throughout the industry support the opinion that the data here recorded are fully representative.

PACKING PROCEDURE

A brief description of the procedure generally followed in canning sardines in Maine is necessary in order that the significance of the materials examined and of the various sampling points be more readily apparent.

The Maine sardine *(Clupea harengus)* is a member of the herring family. The fish are taken in two ways, either by a purse seine or in a weir, which is a large, permanent, maze-like trap from which escape is not easy. They must be held alive for a sufficient length of time to permit their digestive tracts to become free of food material. When free of food, the fish are transferred to a boat; they are salted throughout the loading by throwing crude salt over them. This primary salting serves two purposes: it acts as a preservative in the event a long haul is necessary, and it initiates the pickling process.

Upon arrival at the factory the fish are inspected by a state inspector. If approved by him, sea water is pumped into the hold to facilitate unloading, the fish are hoisted from the hold in a large bucket, and are flumed in sea water to a pickling tank. Just ahead of the tank they pass over a screen which separates them from the flume water.

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The pickling tanks, which are usually of wooden construction, contain a sea-water brine. The strength of the brine and the length of time the fish are held in it depend upon the size of the fish and the operating schedule of the factory. Large fish or fish which must be held longer than the minimum time necessary for adequate pickling require a stronger brine than small fish or fish which can be handled promptly. The holding time in the tank ranges from some four or five to as much as 24 hours, depending upon the empirical judgment of an experienced individual as to when proper salting has been accomplished.

From the pickling tank the fish are flumed in sea water into the buckets of a conveyor belt from which they are deposited into the compartments of the distributing drum of the flaker. The flaker is a machine which has as its function the more or less even distribution of fish on galvanized wire screens known as flakes. The loaded flakes are placed in movable racks and transferred, in lots of 30 to 40, to a steam chamber where the fish are cooked in flowing steam at atmospheric pressure for eight to 12 minutes, the time again being dependent upon the size.

From the steam chamber the racks are transferred to a tunnel dryer through which air at a temperature of 32.2 to 63.3° C. (90 to 146° F.) is passed countercurrent to the steamed fish and in which the fish are held until sufficiently dry. With small fish there may be a slight removal of internal moisture, but in the main the hot air serves merely to dry the surface and thereby make it possible to pack the fish rapidly without crushing them.

After removal from the dryer the racks are held until the fish are cool enough to handle by the packers. The flakes are distributed by conveyor to the packing tables where the packers cut off the heads, and frequently the tails, with shears and place the trimmed fish in the can. The filled cans are placed on trays, or racks, which hold 24 cans. These are stacked with wooden separators, about one-half inch square in cross section, between the trays. The stacks of trays are transferred from the packing table to the sealing machine where approximately 20 ml. of vegetable oil is added to each can and the cans are sealed. The sealed cans drop upon a belt conveyor which carries them to the retort for processing. With few exceptions processing is done under pressure in retorts holding about 1,500 cans. The time-temperature combination used is not uniform throughout the industry. An average would be in the neighborhood of 75 minutes at 107.2° C. (225° F.).

After processing the cans may be water-cooled in the retort or they may be emptied onto a conveyor, carried through a hot detergent spray to remove external oil, and be air-cooled on a slatted table.

BACTERIOLOGICAL STUDIES

Materials and Methods: The bacteriological procedures and media used in this work were adapted from those which have been developed by the Research Laboratory of the National Canners Association for other products. The sampling points followed the sequence of operations and were from locations considered to be potential foci of contamination. The samples were examined for total aerobic bacterial load, for anaerobes, and for resistant spores.

On a considerable number of samples, cultures on 12 per cent sodium chloride agar were included but no significant numbers of halophilic bacteria were ever found and this phase of the work was eventually discontinued. Early results indicated the absence of thermophilic bacteria from the materials examined and the test for these organisms was likewise discontinued. None of the results of tests for either halophilic or thermophilic bacteria has been included in this report. The media used included two per cent tryptone agar (pH 6.8 to 7.0) for quantitative aerobic plating and for stratifying anaerobic tubes, and liver infusion broth over particles of liver tissue, Cameron (1936), for anaerobes. All data are on cultures in these media incubated at 30°C.(86°F.) for three days.

In all instances samples from fish included tissue and, up to the point where the fish were trimmed incident to the packing process, the viscera also. The tissue sample was prepared by cutting out under as nearly aseptic conditions as possible approximately 10 grams of tissue, including skin, from the central area of the body. This was added to 50 ml. of sterile water in a large tube, macerated with a sterile glass rod, and suitable aliquots were cultured. The tube was then steamed at atmospheric pressure for 20 minutes and recultured in order to detect heat-resistant spores. The viscera were dissected out and treated in the same way as the tissue sample. Water and brine samples were cultured in the usual manner. For chips from the wooden surfaces of canning equipment 10 grams of surface shavings were shaken thoroughly with 100 ml. of sterile water and the supernatant liquid cultured. Scrapings of solids from other surfaces were not ordinarily available in quantities as great as 10 grams and consequently it was necessary to use a smaller quantity for the initial dilution.

The results have been calculated to the number of bacteria per gram or per milliliter. This method of presenting the results does not give a rigidly accurate picture of the total number of bacteria per fish since the principal source of the organisms was most probably the surface, and a portion of the sample consisted of subcutaneous tissue. However, comparative results were of primary interest, and by keeping the area dissected out as uniform in size as possible, the procedure was satisfactory for our purpose.

Not all of the data that were recorded have been incorporated in the report. As a general rule the counts given include the highest that was noted and some that might be considered as average or typical. Since the differences between the average and the highest bacteriological findings were ordinarily not very great this selection of data would appear to be appropriate. In most instances no heat-resistant forms were found and much of these negative data have been omitted from consideration. For a few samples which, on the basis of experience with other products, might be considered as having been collected from especially significant points, mention of the results from heated material has been included.

Results: Fish collected at the weir were iced in sterile jars and cultured promptly upon arrival at the laboratory some two to three hours later. The total aerobic count on tissue ranged from 20 to 65 and on viscera from five to 30 per gram. No anaerobes were found. These total

counts are slightly lower than those which were ordinarily obtained from fish handled under commercial conditions, as may be seen by a comparison with the averages of results from several samples collected from five different boatloads at the time of delivery to the factory (Table 1).

The increases in counts were not great enough to cause any concern from the standpoint of quality deterioration and, in the absence of heat-resistant spores, have no potential spoilage significance.

Possible sources of organisms found in or on these early samples of fish include the sea water, the wood of the boat hold, and the salt. A count on sea water collected at a weir showed 100 bacteria per milliliter. Wood chips were taken from the hold of only one boat and on this one sample a

TABLE 1

Average Number of Bacteria Per Gram on Fish at Time of Receipt at Factory

Sample No.	Tissue		Viscera		Remarks
	Aerobes	Anaerobes	Aerobes	Anaerobes	
1	100	28	130	5	17 hours in transit; heavily salted.
2	80	0	39	0	3¾ hours in 10% brine.
3	100	40	20	0	12 hours in ice.
4	625	6	6	55	Lightly salted.
5	20	60	8	4	No information on time in transit or salting.

count of 400 per gram was obtained. The salt added at the time of loading goes into solution in the water on the wet fish. Counts on the brine so formed ranged from 750 to 4,000 per milliliter. Heat-resistant spores and anaerobes were absent from all of these samples. In view of these findings it is probable that the slight increase in numbers of bacteria found on the fish handled under commercial conditions was not because of a multiplication of those originally present but was due to a pickup from the brine. This opinion is supported by the results, presented in a later section, of experiments on the effect of salt concentration on the growth of the bacteria occurring naturally on the fish.

Cultures of the harbor water used in fluming the fish, and wood and brine from the pickling tanks, yielded the results given (Table 2).

The relatively high counts on two wood and two brine samples suggested the possibility of an increase in the bacteria on the surface of the fish as a result of pickling, but repeated examinations failed to reveal any significant differences from the results obtained on fish taken directly from the boat. The results given in the following tabulation show that there is little or no pickup from the tank, and that the bacteria which are present are largely eliminated by the steaming and drying operations (Table 3).

Scrapings were collected from the flaker at various points with which the fish come in contact and from a number of flakes. These were free of

anaerobes and resistant spores but showed on some occasions moderately high total counts. In no instance, however, were the results of such nature as to suggest a spoilage hazard.

The counts on samples held for a prolonged time after drying indicate that the dry fish is not a suitable medium for the development of such bacteria as survive steaming and drying. Changes not referable to bacterial activity and which cause a loss of quality might possibly take place, but bacterial spoilage would not appear to be a problem when for any reason the dried fish cannot be packed as promptly as is customary.

Some increase in numbers of bacteria would be expected as a result of the handling and trimming incident to placing in the can. The increase

TABLE 2

Results of Examination of Harbor Water and Wood and Brine From Factory Pickling Tanks

Sample	Aerobes	Anaerobes
Harbor water—count per ml.		
Low tide	540	Many
Half tide	80	Many
High tide	8	0
Wood chips—count per gram		
Sample 1	1,200	6
Sample 2[1]	5,200	0
Sample 3	4,500	0
Sample 4	10	0
Brine—count per ml.		
Sample 1	7,000	15
Sample 2	340	0
Sample 3	100	4
Sample 4[2]	5,500	0

[1] Wood sample No. 2 showed 50 aerobic spores per gram. [2] Brine sample No. 4 showed 25 aerobic spores per ml.

noted was not very great in absolute numbers except in isolated instances. Counts ranged from as low as 15 to as many as 2,300 per gram with most of the samples falling within the range of about 150 to about 300. Anaerobes were recovered from only two samples and were present in these in very low numbers.

Cultures from surface scrapings of packing tables suggested that an important source of the bacteria in these samples was the table surface. The counts from ordinary hardwood tables were uniformly high with as many as 700,000 per gram being recorded. It should be emphasized that these counts were not the consequence of a lack of physical cleanliness or an indication of an unsanitary condition since the tables were kept scrupulously clean and were thoroughly scrubbed with a detergent solution at the end of each packing period. The entire operation was, furthermore, under continuous supervision by a state inspector.

The knowledge that wooden surfaces may become impregnated with bacteria is, of course, not new. It was early established in connection with work on peas by Cameron, Williams, and Thompson (1928), and the observation made at that time has been repeatedly confirmed. In the vegetable

canning branch of the industry where spores of established spoilage significance may contaminate the product the problem is somewhat more serious than in the present instance where the organisms found were of low resistance to heat and, consequently, could not be considered as constituting a spoilage hazard for the canned product, even if taken into the can in large numbers. The problem has very generally been met in a fully satisfactory way by the substitution, wherever possible, of impervious materials for wood surfaces which come in contact with the product or a moist ingredient of the product. A similar substitution of material for packing tables would minimize the possibility of a pickup of bacteria from the table by the fish. The packing tables are generally plain hard-

TABLE 3

Bacteria Per Gram on Fish From Pickling Tanks, Steamer, and Dryer

Sample	Tissue		Viscera		Remarks
	Aerobes	Anaerobes	Aerobes	Anaerobes	
From tank					
1	110	3	0	0	1¼ hr. in tank.
2	330	0	16	4	12 hr. in tank.
3	35	0	0	0	No record of time in tank.
From steamer	2	7	2	1	Average of 13 samples.
From dryer					
1	8	0	10	26	Average of 8 samples.
2	4	4	2	0	Average of 10 samples.[1]

[1] Held at room temperature for 50 hours after drying and before culturing.

wood, but it was possible to secure samples from a few with masonite and a few with varnished hardwood surfaces. The count on scrapings from these tables was in the range of 2,000 to 3,500 bacteria per gram. The counts on fish from cans filled at these tables were consistently lower than from cans filled at the other type of table.

The sticks used as separators are a doubtful source of bacteria since they are rarely in contact with the fish in the filled can. Examinations were made, however, of shavings from a number of these. Considerable variation was noted, the counts ranging from as few as five to as many as 2,700 per gram.

Although the cultural results from unprocessed material indicated the improbability of bacteria being recovered from processed cans, a large number of such cans were cultured, both promptly after processing and after incubation. Bacteria developed in a low percentage of the cultures, but in view of the observations on air contamination reported by Williams and Clark (1942) in connection with their examination of canned salmon no significance can be attached to these positive cultures. No check on the extent of air contamination similar to that which they made was done, but much of the culturing was done under less favorable environmental conditions than they report, and the results were comparable to those that they obtained. No organoleptic evidence of spoilage was encountered in any instance.

Certain of the results noted in earlier studies on sardines as well as during this work were considered to illustrate the restraining action of salt on bacterial growth. In connection with the handling of the raw product it is important to have some information on salt inhibition of the bacteria which occur naturally on the fish. From work with other products it was known that as little as three per cent of salt would have marked inhibitory action on some organisms. The brine used on sardines has other functions than the inhibition of bacteria, and a brine as weak as three per cent would probably never be used. However, since our primary interest was in the effect on bacteria, it was decided to start at this level a series of experiments to check the inhibitory effect of salt on the growth of the

TABLE 4

Effect of Brine Strength on Multiplication of Bacteria of Natural Occurrence on Fish

Sample No.	Tissue		Viscera		Remarks
	Aerobes	Anaerobes	Aerobes	Anaerobes	
3% brine					
1	70	21	110	5	Initial sample.
2	150	0	27	0	Held for 5 hours.
3	850	50	790	26	Held for 22 hours.
8% brine					
1	33	130	1	10	Initial sample.
2	140	0	31	0	Held for 24 hours.
3	60	0	3	0	Held for 48 hours.
4	45	0	80	0	Held for 72 hours.
5	175	0	620	0	Held for 96 hours.

organisms normally present. Some multiplication of the bacteria was evident in this strength of brine. Later experiments established that a salt concentration of eight per cent was necessary for inhibition. With this strength of brine no significant change in numbers occurred during an extended holding period at room temperature; representative results are given (Table 4).

Some multiplication in the eight per cent brine appeared to have taken place after 72 hours, but no practical importance can be attached to this fact since the holding time is far in excess of that which is compatible with satisfactory commercial quality.

SUMMARY

The results indicate that only a small number of bacteria was normally present in and on the fish and that heat-resistant types were absent. Organisms capable of growing under anaerobic conditions occurred irregularly and were not numerous in any instance. There appeared to be an insignificant pickup of bacteria as a result of the primary salting, but the steaming and drying operations reduced the number to a very low level. Incident to the trimming and filling operation there was a substantial percentage increase in the bacterial load. This increase appeared to be closely related to the nature of the packing table surface, since fish handled on a table with a relatively impervious surface showed fewer bacteria than those from an ordinary hardwood surface.

Thermophilic bacteria were not encountered at any stage of the operation, and halophilic bacteria were present only in low numbers.

A concentration of eight per cent salt was found to be necessary for the inhibition, over a commercially practicable holding time, of the bacteria of natural occurrence on the raw fish.

The survey failed to demonstrate any spoilage hazard in the canning of sardines as done in Maine. This of course does not mean that it is not possible for a hazard to develop or occur, but it affords an explanation of why spoilage difficulties are of infrequent occurrence in the industry.

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EATING QUALITY AND SOME ASPECTS OF COMPOSITION OF TURNIP GREENS AT SUCCESSIVE STAGES OF GROWTH[1]

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Turnip tops are the most popular kind of greens in the South and a prominent food in the southern diet. They well deserve to be, on both nutritional and economic grounds. Their value as a source of certain minerals and vitamins is recognized. The edible portion contains about 26 parts per million of carotene and, according to Fraps and Meinke (1942a), are among the cheapest sources of vitamin A potency for Texans. With an average of 162 milligrams per 100 grams of ascorbic acid in turnip greens as purchased, Floyd and Fraps (1939), they are one of the cheapest sources of this vitamin, Fraps and Meinke (1942b), despite loss in cooking. The present study is concerned with the ordinary chemical composition and the calcium, phosphorus, and iron contents of turnip greens.

The preferred stage for marketing turnip greens and for use out of the home garden is usually described as "young and tender." But at how early a stage of growth does the composition of turnip greens justify their use as food? How much does composition change with age? What change in tenderness occurs as the plants grow? Is palatability related to stage of growth? Such considerations prompted this study.

EXPERIMENTAL PROCEDURE

Samples of Seven Tops turnip greens were harvested at different stages of growth. Portions were submitted to chemical analysis, used for objective tests of tenderness, and judged for palatability after cooking. The greens were grown under 16 different fertilizer treatments in factorial arrangement on Lufkin fine sandy loam at College Station, Texas, in the fall of 1939 and of 1940. These plantings were made primarily for another

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experiment, but they also furnished material for this supplementary study. The youngest plants used were those taken for thinning, while more mature samples came from the guard rows. Successive samples were always taken from the same fertilizer treatment and rows for a specific use. Some of the eating tests were made in homes at College Station, some in the home economics laboratories [2] at the University of Texas, at North Texas State Teachers' College, and at Texas Technological College.

The three samples taken for thinning were smaller than usual market size. The leaves, including stems, in the first of these samples ranged from one and one-half to five and one-half inches in length, and of the third from three to six and one-half inches. The first two samples from the 1940 crop were thinnings, the first having leaves one and one-half to four inches long, the second, two to seven inches. The random samples of marketable size which followed had leaves three to five inches long in the smaller plants and 10 to 16 inches in the larger ones. The last sample consisted of plants which had revived after severe cold weather. These last plants were beyond marketable stage with the larger leaves approximately 12 to 19 inches long, stems thicker and heavier, and vascular bundles stronger than in the preceding sample. Such greens would not ordinarily be chosen to eat.

All greens subjected to chemical analysis were guarded against contamination from dust and metal utensils. The greens were harvested with enough of the root crown to hold the leaves together. The leaves with stems attached were broken off with thumb and fingers, in younger plants about three-fourths inch above the crown, in older plants at about one to one and one-half inches, all leaves suitable for cooking being retained. The same person performed this task with all samples thus minimizing variation owing to the personal factor in handling the fresh greens. The leaves were washed several times in tap water until free from sand. Greens for chemical analysis were rinsed twice in distilled water and spread out in a single layer on cheesecloth supported on a special rack. Water on the surface of the leaves was removed by gently patting with cheesecloth, aided by draft from an electric fan. Weight of washed leaves, when dry but not wilted, was used as "fresh weight," since the leaves in this state more nearly approximated uniform turgidity than at any other point in handling them. The leaves were then allowed to wilt and were dried at 100°C.(212°F.) in an electric oven with forced draft. They were ground in a ball mill and sifted through a 30-mesh silk bolting cloth which was held by the hands or fastened in a wood frame.

Two or more determinations of each constituent were made on each sample. Chemical analysis included determination of water content of the fresh greens and of protein, fat, crude fiber, nitrogen-free extract, total ash, calcium, phosphorus, and iron contents of dried samples. Iron determinations were made by a procedure based on the o-phenanthroline method of Saywell and Cunningham (1937). However, nitric acid as well as sul-

[2] The authors make grateful acknowledgment to Miss Jennie Wilmot, Miss Marjorie Acheson, and Miss Jonnie McCrery who respectively supervised the cooking and judging of the greens at the University of Texas, North Texas State Teachers' College, and Texas Technological College.

furic and perchloric acids were used in the wet ashing of the samples, according to the method of Jackson (1938), and an Evelyn (1936) photoelectric colorimeter was used for measuring color concentrations. For all other constituents methods of the Association of Official Agricultural Chemists (1940) were used.

Tests for tenderness were made on six successive samples of greens from the 1940 crop by means of a Warner-Bratzler shearing device. A cardboard ring of 1.9 centimeters diameter was filled with the stems of the greens held parallel to one another and extending approximately one and one-half inches through the ring. Several such bundles were used for the test of each sample. The first shear was made on the bundle of stems about one-half inch below the ring toward the stem ends. Successive shears followed at points from one to two or three inches in progressive order toward the tip of the leaves so that shearing strength was determined for stems, stem portion with lower lobes of leaf blade, and leaf blade. After the first shear the bundle was held together only by the hand of the operator.

The greens cooked by College Station families were cleaned, washed, and weighed in the laboratory, the same procedure being followed as for greens to be analyzed except rinsing in distilled water and removal of surface water. Each household followed its own custom and reported the procedure used. Each adult person who ate the greens, which were served as part of a regular meal, filled out a judging record. Four families in the scientific and two in the clerical staff of the Experiment Station, one farm laborer's, and a colored maid's took part in the cooking tests, making a total of 24 persons judging the eating qualities of the greens.

Greens to be cooked and judged in the home economics laboratories were wrapped in moisture-proof Cellophane as harvested, packed into heavy paper boxes, and shipped at once by express. Transportation required about 24 hours. During any interval between arrival and cooking, the greens were held in the refrigerator in the original shipping package. Each laboratory cooked three-fourths-pound samples of washed greens (leaves with stems) plus one-half teaspoon of salt in one-half, one, three, and six cups of tap water, and three-fourths-pound samples each of stems and leaves separated, in three cups of water. Cooking water was boiling when greens were added. Each laboratory chose whether to use open or closed pan (except that all used cover with the sample cooked in one-half cup of water). The "done" stage was judged by piercing with knife or fork or trying with the teeth. Record was made of cooking time and of the number of servings including both greens and liquor from each sample cooked. Judging records were filled out by 38 students and instructors; from four to 19 persons participated in judging each sample.

RESULTS AND DISCUSSION

Chemical Analyses: Data from the chemical analyses are recorded (Table 1); of the total 112 average values obtained for the dried greens,[3] 31 of them are averages of the results from the two chemistry laboratories.

[3]Appreciation is expressed to J. F. Fudge for analyses of dried samples made in the Texas Agricultural Experiment Station.

TABLE 1

Composition of Seven Tops Turnip Greens at Successive Stages of Growth

Harvest date	Sample		Moisture-free basis								Fresh basis								Harvest date
	Source	Age[1]	Protein	Fat	N-free ext.	Fiber	Ash	Ca	P	Fe	Water	Protein	Fat	N-free ext.	Fiber	Ash	Ca	P	Fe	
		days	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	
1939 Crop																			
Dec. 12,'39	Fertilized guard rows thinning	40	42.00	5.22	27.48	6.68	14.95	1.45	.870	.0154	91.63	3.53	.44	2.30	0.56	1.25	.12	.073	.0013	Dec. 12,'39
Dec. 20,'39		48	38.79	4.65	30.80	6.79	14.09	1.73	.712	.0196	90.64	3.63	.44	2.88	0.64	1.32	.16	.067	.0018	Dec. 20,'39
Jan. 16,'40		75	35.64	3.80	39.05	6.15	11.36	1.58	.702	.0192	87.46	4.47	.48	4.90	0.77	1.42	.20	.088	.0024	Jan. 16,'40
1940 Crop																			
Oct. 10,'40	Thinning entire planting	26	43.84	3.50	26.24	7.52	18.90	3.38	.571	.0159	92.33	3.36	.27	2.01	0.58	1.45	.26	.044	.0012	Oct. 10,'40
Oct. 23,'40		39	42.46	4.14	25.29	8.86	19.24	3.13	.672	.0212	92.87	3.03	.30	1.80	0.63	1.37	.22	.048	.0015	Oct. 23,'40
Oct. 23,'40	Unfertilized guard rows	39[2]	39.39	3.50	26.42	9.77	20.92	3.73	.511	.0231	93.82	2.43	.22	1.63	0.60	1.29	.23	.032	.0014	Oct. 23,'40
Oct. 30,'40		46	37.19	3.28	27.52	11.25	20.76	3.26	.612	.0216	93.44	2.44	.22	1.80	0.74	1.36	.21	.040	.0015	Oct. 30,'40
Nov. 6,'40		53	37.58	2.51	30.18	11.24	18.49	2.80	.746	.0201	92.27	2.90	.19	2.33	0.87	1.43	.22	.058	.0016	Nov. 6,'40
Nov. 13,'40		60	33.04	3.00	39.43	10.03	14.50	2.30	.570	.0151	89.29	3.54	.32	4.22	1.07	1.55	.25	.061	.0016	Nov. 13,'40
Dec. 11,'40		89	34.32	2.54	39.04	10.56	13.54	2.22	.649	.0200	89.46	3.62	.27	4.11	1.11	1.43	.23	.068	.0021	Dec. 11,'40
Oct. 30,'40	Fertilized guard rows	46	40.43	3.60	25.37	11.12	19.48	2.65	.777	.0201	93.92	2.46	.22	1.54	0.68	1.18	.16	.047	.0012	Oct. 30,'40
Nov. 6,'40		53	40.63	2.94	28.30	10.82	17.30	2.20	.871	.0204	92.05	3.23	.23	2.25	0.86	1.38	.17	.069	.0016	Nov. 6,'40
Nov. 13,'40		60	35.82	3.88	35.96	9.80	14.54	1.91	.718	.0160	89.35	3.81	.41	3.82	1.04	1.55	.20	.076	.0017	Nov. 13,'40
Dec. 11,'40		89	36.37	3.60	34.83	11.03	14.18	1.98	.722	.0165	90.10	3.60	.36	3.45	1.09	1.40	.20	.071	.0016	Dec. 11,'40

[1] From planting to harvest. [2] Thinning.

The data on the moisture-free basis indicate that the protein, ash, calcium, and iron tended to decrease with increased growth, while the nitrogen-free extract increased as did also the crude fiber with some irregularity. Phosphorus and fat content varied irregularly.

The data expressed on the fresh basis present a more consistent relationship between stage of growth and composition. Trends are in the same direction among the samples of the two years. In general, content of water decreased progressively, while all other constituents increased with age; however, the general trend in the 1940 series (Table 1) is reversed by the thinning (youngest) samples for some constituents, by the oldest sample for others. The lowest content of phosphorus is in the second sample instead of the first in the 1939 series, and for calcium in the unfertilized series of 1940.

Neither the data nor records of handling the greens provide satisfactory explanation of the instances of departure from the general trend of change in composition of fresh greens as the plants grew. Some common factor must have been operating since the same samples—only the oldest and the two harvested by thinning in 1940—were involved in divergence from the general trend, except for phosphorus in the 1939 series and calcium in the unfertilized series of 1940 as noted above.

How important with respect to the food value of turnip greens are the changes in composition that occur with growth of the plants? A final answer to that question must await further research to determine the extent to which the human body can utilize the nutrients of turnip greens when eaten as part of a well-selected diet. But a partial and tentative answer is offered in the estimation that has been made of the dietary contribution from one serving of turnip greens. For this estimation the data for the fresh greens have been used since they represent the greens as they are when ready to cook. It has been assumed, as suggested by the cooking tests of this study, that 100 grams of fresh raw greens approximate one serving when cooked, also that all liquor is eaten, and that the nutrients are available to the human body. Thus considered, turnip greens have been shown by this study to be well worth eating at every stage of growth as a source of calcium, iron, and crude fiber, but to be of little consequence for protein, fat, nitrogen-free extract, and phosphorus in an otherwise good diet.

The calculated contributions and aids in comparing certain of them with the current recommended daily allowance for a moderately active grown man are shown (Table 2), Food and Nutrition Board, National Research Council (1943) and Sherman and Lanford (1940).

The greens with highest calcium content would furnish 0.26 gram of calcium in one serving which is 32.5 per cent of the recommended daily allowance (0.8 gram) for an adult, while the sample containing the least calcium would supply in one serving 0.12 gram of calcium or approximately 15 per cent of the allowance. Of iron, one serving of the best sample would supply 0.0024 gram, which is 20 per cent of the recommended daily adult allowance (0.012 gram); the poorest sample would furnish

0.0012 gram, or 10 per cent of the allowance. Considering that the iron in the usual well-selected mixed diet must be secured from a number of sources, the authors believe that any food, one serving of which furnishes as much as 10 per cent of the daily allowance of iron, should be considered valuable and even smaller contributions are not to be disregarded. Fiber values ranged from 0.56 to 1.11 per cent, making these turnip greens at every stage of growth comparable with other leafy vegetables which are depended upon to give bulk to the diet.

Total ash in one serving of these greens ranged from 1.18 to 1.55 grams expressed on the fresh basis, values comparable with other foods prized for a mixture of minerals. In these samples of extreme ash values the calcium, phosphorus, and iron together constituted 13 per cent (minimum) and 22.6 per cent (maximum) of the total ash; hence, turnip greens should be of value also for other essential minerals.

TABLE 2

Contributions to the Diet From One Serving[1] of Turnip Greens

(Total series of 1939 and of 1940 considered)

Constituents	From samples		Recommended allowance[2]	Proportion of allowance in samples		Calories	
	Min.	Max.		Min.	Max.	Min.	Max.
	gm.	*gm.*	*gm.*	*pct.*	*pct.*		
Crude fiber	0.56	1.11	.....	.....	.....	.....	.....
Ash	1.18	1.55	.....	.....	.....	.....	.....
Calcium	0.12	0.26	0.8	15.0	32.5	.....	.....
Phosphorus	0.032	0.088	1.32	2.4	6.7	.....	.....
Iron	0.0012	0.0024	0.012	10.0	20.0	.....	.....
Protein	2.44	4.47	70.0	3.5	6.4	9.8	17.9
Fat	0.19	0.48	.....	.....	.....	1.7	4.3
N-free extract	1.54	4.90	.....	.....	.....	6.2	19.6
						17.7	41.8

[1] One hundred grams fresh, about one-half cup cooked. Assume all liquor eaten and nutrients available to human body. [2] For a moderately active adult man.

One serving of these greens could have furnished but 2.4 to 4.5 grams of protein, which is unimportant compared with a total daily allowance of 70 grams for an adult man and 80 to 100 grams for adolescents. Of fat, one serving would yield only 0.19 to 0.48 gram; of nitrogen-free extract, from 1.5 to 4.9 grams, only part of which is digestible carbohydrate. The maximum nitrogen-free extract and fat together would supply less than one per cent of the energy of a 3,000-Calorie diet in comparison with the usual recommendation that fat and carbohydrates supply from 85 to 90 per cent of the total daily energy. The sample of greens richest in phosphorus would furnish only 0.088 gram and the poorest sample 0.032 gram, unimportant quantities when expressed as 6.7 and 2.4 per cent of the recommended daily amount of 1.32 grams of phosphorus.

Tenderness Tests With a Shearing Device: The slow rate of increase in shearing strength and the nearly uniform values for each of the three parts of the greens at the same age (Table 3) are surprising features in these objective tests of tenderness. The total range of shearing strength was 12.75 to 21.97 lb. for stems; 12.94 to 21.42 lb. for stems with leaf

TABLE 3

Shearing Test on Turnip Greens

Sample		Harvest date, 1940	Shearing strength (lb.) of 2.84 sq. cm. area [1]								
			Stems			Stems with leaf lobes			Leaves		
Source	Age,[2] days		Number shears	Pounds		Number shears	Pounds		Number shears	Pounds	
				Range	Av.		Range	Av.		Range	Av.
First thin, whole planting	26	Oct. 10	9	11.00-15.00	12.75	....	.....		8	9.00-12.50	11.22
Second thin, whole planting	39	Oct. 23	16	11.75-16.00	13.23	9	11.75-14.50	12.94	17	10.00-16.00	12.01
Guard rows not fertilized	46	Oct. 30	21	11.50-16.50	14.07	4	12.00-16.50	14.81	23	11.50-16.25	14.02
	53	Nov. 6	24	13.75-17.75	16.05	8	14.00-20.75	17.06	24	12.00-17.50	14.95
	60	Nov. 13	27	12.50-21.75	17.89	15	14.00-24.50	19.25	25	15.75-21.50	17.93
	89	Dec. 11	26	17.00-27.25	21.97	13	16.75-27.00	21.42	28	16.00-27.00	19.64

[1] Cardboard ring, diameter 1.9 cm., used to hold the bundle. [2] From planting to harvest.

lobes; and 11.22 to 19.64 lb. for leaf blades. Samples collected by thinning, consisting of such plants as should certainly be used from the home garden although too small to market, compared with the greens which first reached marketable size, showed less difference in shearing strength for stems (1.3 lb., 9.3 per cent) and stems with leaf lobes (1.9 lb., 12.6 per cent) than for leaves alone (2.8 lb., 19.9 per cent). The toughness of marketable greens increased in two weeks to the extent of 3.8 lb. (27.1 per cent) shearing force for stems, 4.4 lb. (29.9 per cent) for stems with leaf lobes, and 3.9 lb. (27.8 per cent) for leaf blade only. The last sample in the series, a month older than the preceding sample and beyond marketable size and age, sheared at an increase over the preceding sample of 4 lb. (22.8 per cent) for stems, 2.2 lb. (11.3 per cent) for stems with leaf lobes, and 1.8 lb. (1.0 per cent) for leaves. These results of tests of tenderness are contrary to the common opinion that stems of older greens are very much tougher than the leaves, for in the first five samples the stems required from 0.04 lb. (marketable greens) to 1.5 lb. (thinning samples), average 0.8 lb., more of shearing force than did corresponding leaves and in the oldest sample only 2.3 lb. more. However, the leaves may contain materials which sometimes become more tender upon cooking, as the laboratories scored six of nine samples of cooked leaves slightly higher (more tender) than the corresponding samples of stems (Table 5).

Cooking and Eating Tests: From one to six families at College Station cooked and ate not only samples harvested the same day on which greens were taken for chemical analysis, but also other samples harvested on intermediate dates from the 1940 crop. The home economics laboratories cooked and judged only samples of marketable size greens except that one of them used also the last thinning sample. The results of the eating tests are given (Table 4) for the College Station families and (Table 5) for the laboratories.

The cooked greens were judged by use of the following scale of values for each of three qualities:

Tenderness	Degree of bitterness	Characteristic turnip greens flavor
5 = very tender	5 = absent	5 = excellent
3 = tender	5 = present, but agreeable	3 = good
2 = neutral	3 = present, slightly objectionable	2 = fair
1 = tough	2 = more objectionable	1 = poor
0 = too tough to eat	1 = very objectionable	0 = not acceptable
	0 = refused to eat	

The scoring scale in preliminary use assigned the five-point value to "bitterness absent." Some judges, among them several home economists, objected, saying turnip greens should be slightly bitter, and that's the way they preferred them! Hence, the provision for choice on five-point value for bitterness.

Variations in the average scores by families (Table 4) appear to be in no way related to stage of growth. All scores are 2 or above (fair quality and better) with exception of one rating of 1 (very objectionable) given to bitterness. That this low score is not related to age of greens is

TABLE 4

Summary of Reports of Eating Tests of Turnip Greens by College Station Families

Date of harvest, 1940	Date cooked	Number families	Number judges	Ratings			Notes
				Tenderness	Bitterness	Flavor	
				Range in score card			
				5 to 0	5 to 0	5 to 0	Out of 76 judgments on each factor of eating quality, "0" was given tenderness once, bitterness twice, and flavor twice.
				Average of given score			
Oct. 10[1]	Oct. 12 & 13	3	9	4.55	3.67	3.55	
Oct. 23[1]	Oct. 26 & 29	2	5	3.80	2.80	3.20	Cooking time range = 13 to 52 min. (except 1 family, Oct. 31, = 1 hr.)
Oct. 31[1]	Nov. 4	2	3	2.67	4.33	2.67	
Nov. 5	Nov. 7	1	1	2.0	3.0	3.0	
Nov. 5	Nov. 9	1	1	3.0	5.0	5.0	Shearing strength range = 11 to 14 lb.
Nov. 6[1]	Nov. 6, 7, 8, & 10	5	12	2.67	4.17	3.42	
Nov. 7	Nov. 7	1	2	2.0	5.0	2.0	
Nov. 12	Nov. 12	1	2	3.0	3.0	2.0	Cooking time range for all harvesting:
Nov. 13[1]	Nov. 14, 15, & 16	6	15	3.13	3.00	2.80	November 6 and thereafter = 1 to 2 hr., 5 min., except:
Nov. 13	Nov. 13	1	2	2.0	5.0	5.0	1 family, Nov. 13 = 45 min.
Nov. 13	Nov. 15	1	2	2.0	1.0	2.0	1 family, Nov. 19 = 15 min.
Nov. 19	Nov. 21 & 25	2	3	4.33	3.33	2.67	1 family, Dec. 11 = (40″ leaves and stems) (44″ leaves only).
Nov. 22	Nov. 22	1	2	3.0	5.0	3.0	
Nov. 27	Nov. 27	1	2	2.0	5.0	2.0	Shearing strength rose progressively from 16 lb. Nov. 6 to 27 lb. Dec. 11.
Nov. 27	Nov. 28	1	2	3.0	5.0	2.0	
Dec. 11[1]	Dec. 14, 15, & 16	5	13	3.46	3.08	3.23	Plants revived after severe cold weather.

[1] On these dates, samples were taken for complete analysis.

TABLE 5

Summary of Eating Tests of Turnip Greens by Three Home Economics Laboratories

Harvest date, 1940	Date cooked	Institution	Number judges	Average ratings																	
				Leaves with stems												Stems separated from leaves					
				Tenderness				Bitterness				Flavor				3 cups water to cook ¾ lb. greens					
				Water to ¾ lb. greens												Tenderness		Bitterness		Flavor	
				½ c.	1c.	3c.	6c.	½ c.	1c.	3c.	6c.	½ c.	1c.	3c.	6c.	Stems	Leaves	Stems	Leaves	Stems	Leaves
Oct. 31	Nov. 2	Univ. of Tex.	4	0	1	2	3	0	1	4	5	.....	.....	.....	.....	1	0	2	3	.....	.....
Nov. 5	Nov. 7		4	0	2.50	4.00	4.50	2.25	1.00	2.25	4.75	0.50	2.25	3.25	2.50	4.25	5.00	4.50	0	3.00	0
Nov. 12	Nov. 14		4	2.50	3.50	4.00	5.00	1.00	1.00	3.50	5.00	0	0.75	2.25	3.50	3.50	4.75	4.25	3.75	2.75	3.25
Oct. 31	Nov. 6	Tex. Tech. Col.	9	0.56	0.33	1.00	1.78	3.22	2.67	3.88	3.33	1.56	1.44	1.38	1.67	2.67	1.56	4.33	2.89	2.11	1.89
Nov. 7	Nov. 8		7	2.14	2.43	4.14	3.86	3.57	4.14	5.00	5.00	2.14	2.29	3.14	3.14	4.00	3.86	5.00	5.00	2.71	3.71
Nov. 13	Nov. 15		7	2.00	2.00	3.43	4.71	3.57	3.00	3.57	5.00	2.57	1.57	3.00	1.57	2.57	3.57	3.57	4.43	1.86	2.57
Oct. 23	Oct. 25	No. Tex. S.T. Col.	17	3.24	2.88	3.06	4.06	3.12	1.65	2.88	5.00	2.41	1.82	2.18	3.18	.....	.....	.....	.....	.....	.....
Oct. 30	Nov. 1		17	2.18	2.28	2.78	3.39	2.18	2.06	4.33	4.44	1.53	1.78	2.39	2.72	1.89	3.71	2.33	4.06	2.00	2.59
Nov. 7	Nov. 8		19	0.42	1.42	2.95	3.16	2.05	1.89	3.84	4.00	1.32	2.11	2.58	2.68	2.32	3.63	3.53	3.42	2.63	2.89
Nov. 14	Nov. 15		19	2.21	2.28	.....[1]	4.79	2.00	2.28	.....[1]	3.37	1.68	1.67	.....[1]	2.47	2.84	3.74	2.68	3.42	2.42	2.21

[1] Omitted.

indicated by the same family's score of 5 on bitterness given to another sample of greens harvested the same day and also to some harvested nine days and some two weeks later. The youngest sample with a score of 4.55 on tenderness is nearly matched by one of 4.33 for greens five weeks older; the score of 3.67 on bitterness for the younger sample is exceeded (higher score, less bitterness) by that of eight older samples, and the score of 3.55 on flavor of the youngest sample is exceeded by two scores of 5 and is most nearly matched by the score of 3.42 on greens a month older and by 3.23 for the last sample in the series.

As may be seen (Table 5), when three-fourths pound of leaves with stems were cooked in either three or six cups of water the greens were more acceptable than those cooked in one-half or one cup of water. Of the 10 samples cooked and judged, all scores on the lots cooked in three and six cups of water were above 2, except that one sample was graded lower than 2 on tenderness and flavor, and another sample on flavor, by one laboratory. Of 57 scores on leaves with stems cooked in three and six cups of water, 35 of them were 3 or above. Thus the greens were rated high by the laboratories. With scores so nearly the same for the three-fourths-pound lots of leaves with stems cooked in three and six cups of water, respectively, it would seem wise to propose the lesser proportion of water, one quart (four cups), to cook one pound of greens, as the smaller quantity would produce an excellent flavor and an amount of liquor more likely to be entirely consumed than would a larger amount.

Stems and leaves cooked separately in three cups of water to three-fourths of a pound of sample showed small differences in scores which were sometimes in favor of stems, sometimes in favor of the leaves, in each of the three qualities judged (Table 5). Compared with corresponding samples of leaves-with-stems cooked in three cups of water, among seven lots of samples cooked on different days, two of stems only and three of leaves only, received for tenderness a score as high as, or above, the score given leaves with stems. On bitterness, five of eight samples of stems only, and four of eight of leaves only, were scored as high as or higher than leaves with stems from the same harvestings. Flavor scores were higher for three of seven samples of stems only, and five of seven samples of leaves only, than for corresponding samples of leaves with stems. Zero score was given to no sample of the leaves-with-stems cooked in three cups of water nor to stems only, but zero was given to tenderness in one sample and once each to bitterness and flavor in another sample of leaves only.

A few persons objected to the "stringiness" of the cooked stems in older greens; but a greater number made no such comment. Some noted that leaves cooked with stems were less bitter than leaves cooked alone, an observation supported by the laboratory scores. The evidence from this study would justify encouraging the use of turnip greens stems by those who find them palatable. To eat the stems of turnip greens is one way to conserve food value, a special goal in war time. A study is in progress of the composition of separate leaf and stem portions of turnip greens at successive stages of growth.

The small differences in the scores given by the College Station families to greens of different ages are the more striking, considering the variations

in cooking procedure. Six of eight families cooked the greens covered, one without cover, and one family sometimes used the cover, other times did not. Some families used all of the stems, others discarded all stems; some families used all the stems in the younger greens but discarded all or part of the stems in older samples only. One housewife discarded stems of a younger sample, as was her custom, but followed the suggestion to cook them in an older sample, and her family either did not object or positively liked the mixture of stems and leaves. Amount of cooking water to one pound of greens varied from one to eight cups. In one home the water to cook one pound of greens varied from four to eight cups and was not related to age of greens; in another home, from one to three cups of water was used to one pound of greens. Cooking time in homes seemed to be as much a matter of custom as of age of greens. The least difference, in the same home, between cooking time of youngest and oldest greens was 27 minutes, the greatest difference, one hour and 15 minutes. As between homes, cooking time of the youngest greens differed by 39 minutes (range 13 to 52 minutes), while more mature greens from the same harvesting were cooked as much as one hour and 20 minutes longer in some homes than in others. Shorter time of cooking will help to conserve vitamin values; yet the greens must be cooked enough to be palatable, else they are likely not to be eaten.

Despite the great popularity of greens and the fact that but one general type of preparation is used for them—boiling till tender and seasoning with fat or fat meat—the variations in the details of cooking procedure are amazing. The situation is well summed up by the instructor of one of the co-operating foods classes after reading the description given by her 21 students of the details of cooking turnip greens in their own homes: "The one point on which they agree is that the sand, bugs, and spiders are not to be cooked."

The laboratories varied in their use of a cover on the cooking container. One never covered the greens, another used a cover till the greens wilted then removed it, and the third covered the greens in the first two samples (harvestings) but omitted covers from the third sample. Similarly with consumption of the liquor, one laboratory served none of it; a second added about one tablespoonful to each serving of greens; the third served all the liquor if there was only a small amount, or used one to two tablespoonfuls per serving if there was an excess of liquor.

Cooking times of 20 minutes to one and one-half hours were used by the laboratories, but most samples, regardless of amount of water, were tender after 30 to 50 minutes of cooking. Cooking times used for separated leaf and stem samples were similar to times used for leaves with stems from the same harvesting, with one exception—55 minutes for stems only as against 20 to 35 minutes for the other five lots cooked out of the same sample.

The number of servings made by the laboratories out of three-fourths pound of raw washed greens varied from two and one-half to five, with three and four the most frequent numbers. Thus the average size of one serving in a laboratory was near 100 grams of raw fresh greens. Among

College Station families the number of servings calculated to a fresh-weight basis ranged from 54 to 76 grams in the home of a clerical worker, and from 151 to 215 grams in the home of the farm laborer, but this second family used 170 grams per serving in five out of eight cooking tests. Other families had intermediate ranges in size of servings so that 100 grams would approximate the average. Family custom, along with the practice in the laboratories, suggests that 100 grams of raw fresh greens may be used as an average serving.

SUMMARY AND CONCLUSIONS

Turnip greens of Seven Tops variety were grown in two fall seasons at College Station, Texas. Three samples were harvested over a period of five weeks from the first crop and 11 samples over eight and one-half weeks from the second crop.

On the fresh-greens basis there was a decrease in water content but an increase in protein, fat, nitrogen-free extract, total ash, calcium, phosphorus, and iron. Water content averaged 91.3 per cent and maximum change with age was slightly under five per cent. Considering all samples on the fresh basis, the range in percentage content of the solid constituents were as follows: protein 2.43 to 4.47, fat 0.19 to 0.48, nitrogen-free extract 1.54 to 4.90, fiber 0.56 to 1.11, total ash 1.18 to 1.55, calcium 0.12 to 0.26, phosphorus 0.032 to 0.088, and iron 0.0012 to 0.0024.

The cooking tests of this study suggest that 100 grams of the raw washed greens will make an average serving when cooked. Such a serving of these greens would have supplied at different ages from 15 to 33 per cent of the recommended daily adult allowance of calcium, and from 10 to 20 per cent of the iron allowance. At each age studied they would have supplied also valuable amounts of total ash and fiber but would have been of little consequence as a source of protein, fat, carbohydrate, and phosphorus.

The eating quality was not related to stage of growth whether tested by families or foods classes and irrespective of whether stems were cooked or discarded.

Toughness of raw stems, leaf blades, and stems with leaf lobes increased with age as shown by mechanical shearing tests, but at each stage the toughness of these three parts of the greens leaf was similar. Shearing strength ranged from 12.75 to 21.97 lb. for stems, 11.22 to 19.46 lb. for leaf blades, and 12.94 to 21.42 lb. for stems with leaf lobes. Cooking tendered all three parts of the greens in approximately the same time. Cooking time ranged from 13 minutes to two hours and 20 minutes, among families and from 20 minutes to one hour and 30 minutes, in the foods laboratories. Cooking time varied as much with household custom as with age of the greens. It would be advisable to use the shortest time, 15 minutes to one hour, to tender the greens so as to conserve vitamin values.

Greens cooked in three cups (one and one-half pints) or six cups (one and one-half quarts) of water to three-fourths pound of raw greens were rated higher on tenderness, degree of bitterness, and characteristic turnip-greens flavor by the scores of home economics students and instructors than other lots from the same sample cooked in one-half or one cup of

water. It is suggested, therefore, that one quart (four cups) of water to one pound of raw greens be used for cooking since that procedure would insure a palatable product and be more conducive to consumption of all the liquor, than would the use of more water.

This study emphasizes that Seven Tops turnip greens, including all parts, were good to eat from the earliest possible to latest possible harvest date and that they are to be prized at all stages of growth as a source of fiber, total ash, calcium, and iron in the diet.

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CALCIUM, PHOSPHORUS, AND IRON CONTENTS OF TURNIP GREENS AS INFLUENCED BY METHOD OF SAMPLING[1,2]

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Because sampling is one of the most important of research procedures and fresh plant material is very difficult to sample, special interest is attached to the influence of method of selecting representative material upon the results of subsequent chemical analysis. Opportunity has been taken to make such a study by utilizing the guard rows of an experimental planting of Seven Tops turnip greens grown at College Station, Texas, in the fall of 1938. It seemed advisable to determine both precision of analytical results and economy of labor as influenced by the three methods of sampling employed. For purpose of comparison with certain aspects of the results with the samples harvested by different methods, data secured in another study of turnip greens grown the same year at Winter Haven, Iowa Park, and College Station, Texas, are drawn upon for this report.

EXPERIMENTAL PROCEDURE

The entire planting consisted of 20 rows each 40 feet in length. Half the length of each row received a uniform application of the same fertilizer (ammonium sulfate, superphosphate [23 per cent], and muriate of potash at the rate of 60 pounds each per acre). The remaining 20 feet

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[2]A portion of the data reported here was used in the thesis presented by Laverne McWhirter in partial fulfillment of requirements for the degree of Master of Science from Texas Technological College.

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of each row was grown without fertilizer. The outer five rows on either side of the planting were the guard rows, shown by diagram (Fig. 1). The inner four guard rows on either side of the fertilized portion of the planting—Rows 2, 3, 4, and 5 in one set and Rows 16, 17, 18, and 19 in the other set—and the inner four guard rows on one side of the unfertilized portion were used for this study of method of sampling. There was a buffer area of two feet at either end of each harvested row.

Three methods of sampling were employed. In the two sets of fertilized rows random samples were taken in two ways: (1) separately from each of the four rows in each set, one sample of about 2,000 grams; (2) from each set, four composite samples of about 2,000 grams each, made up of

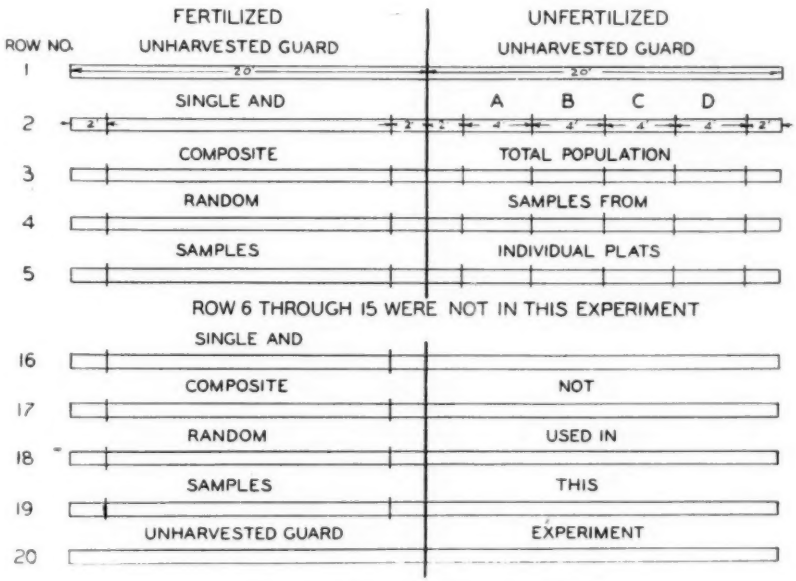

FIG. I. DIAGRAM OF AREAS HARVESTED

equal parts from each of the same four rows which were sampled separately. In the third method the central 16 feet of each of the four unfertilized rows were divided into four-foot plats and all plants in each plat taken as the sample. Thus there were eight random single-row samples, eight random-composite samples, and 16 total-population samples.

Plants were harvested with enough of the root crown to hold the leaves together. All samples were prepared for analysis, and moisture in the fresh greens was determined in the Division of Rural Home Research at College Station, the same procedures being used as described elsewhere.[4] All determinations of content of calcium, phosphorus, and iron in the dried material were made by the same person, a portion of the work having been done in the chemistry laboratory at Texas Technological College, the remainder in the home economics laboratory of the Mississippi Experi-

TABLE 1

Summary of Record at Harvesting and Results of Chemical Analysis

Sample				Weight as harvested	Good leaves used for analysis		Moisture in fresh greens	Minerals, H_2O-free basis		
Description			Row No.		Percentage of sample[1]	Fresh weight, washed		Ca	P	Fe
				gm.		*gm.*	*pct.*	*pct.*	*pct.*	*p.p.m.*
Random samples from fertilized guard rows	Harvested November 14	Single row	2	2181.0	47.98	1048.7	91.69	2.92	.669	165.7
			3	1978.5	44.76	921.0	91.73	2.80	.769	139.1
			4	2220.7	52.52	1214.1	91.77	3.09	.723	149.8
			5	2055.9	45.65	889.0	91.00	2.59	.744	164.5
					Av. 47.73		Av. 91.55	Av. 2.85	Av. .7262	Av. 154.8
		Composite[2]	2, 5,	1974.3	49.75	998.5	92.12	2.68	.730	162.1
				1987.8	50.02	1001.6	92.02	2.92	.717	150.9
			4, 5	1981.8	49.27	962.7	dry wt. lost	2.90	.717	157.8
				2001.7	38.18	899.1	92.72	2.71	.754	145.3
					Av. 46.81		Av. 92.29	Av. 2.80	Av. .7295	Av. 154.0
		Single row	16	1878.7	58.27	1148.1	90.97	3.15	.660	128.8
			17	2134.1	66.54	1528.3	90.95	3.03	.627	124.9
			18	2110.7	58.44	1343.5	91.47	3.14	.610	148.3
			19	2132.0	56.68	1329.0	90.54	3.11	.705	137.5
					Av. 59.98		Av. 90.98	Av. 3.11	Av. .6505	Av. 134.9
		Composite[2]	16, 17,	2031.8	49.41	1059.1	90.88	2.68	.676	135.6
				2061.6	54.53	1302.4	91.53	2.83	.675	131.3
			18, 19	2072.4	51.21	1297.4	92.61	2.68	.698	152.3
				2171.4	48.18	1156.2	91.20	2.58	.720	158.2
					Av. 50.83		Av. 91.56	Av. 2.69	Av. .6922	Av. 144.4
Total population from unfertilized guard rows	Harvested November 15	All plants in each plat of each row		*Yield*						
			2a	2118.4	46.09	1017.4	91.35	2.75	.650	122.2
			2b	1849.9	55.26	1073.2	90.64	2.79	.643	125.6
			2c	1026.5	58.12	664.9	91.07	3.03	.631	122.1
			2d	1479.9	55.55	899.8	90.53	2.90	.601	130.3
					Av. 53.76		Av. 90.90	Av. 2.87	Av. .6313	Av. 125.0
			3a	1856.5	54.84	1064.8	90.98	2.75	.637	104.2
			3b	1947.9	56.51	1166.9	90.92	2.86	.629	101.3
			3c	2476.5	46.42	1233.6	90.95	2.62	.652	101.0
			3d	2002.9	48.65	1047.1	90.92	2.52	.647	104.0
					Av. 51.61		Av. 90.94	Av. 2.69	Av. .6413	Av. 102.6
			4a	2283.4	54.29	1343.4	91.74	2.62	.695	112.2
			4b	1683.6	51.36	926.5	92.10	2.63	.701	109.6
			4c	2202.5	50.13	1194.0	91.42	2.58	.692	103.3
			4d	1604.1	52.30	906.1	90.87	2.78	.652	115.6
					Av. 52.02		Av. 91.53	Av. 2.65	Av. .6850	Av. 110.2
			5a	1888.4	52.75	1050.9	91.20	2.99	.617	126.9
			5b	2095.4	49.44	1129.9	91.73	2.85	.621	113.0
			5c	2420.2	47.07	1187.0	91.14	2.84	.634	113.4
			5d	1928.4	50.51	1001.0	90.60	2.48	.657	116.7
					Av. 49.94		Av. 91.17	Av. 2.79	Av. .6322	Av. 117.5

[1] As harvested. [2] Equal parts from each of the four rows indicated.

ment Station.[5] Iron determinations were made by a procedure based on the o-phenanthroline method of Saywell and Cunningham (1937) and described elsewhere.[4] For moisture, calcium, and phosphorus, methods of the Association of Official Agricultural Chemists (1940) were used. At least two determinations of moisture and of each of the three minerals were made on each sample, but in many instances there were from three to five determinations. The values for each sample for which there were four or more determinations were subjected to a statistical test described by Fales and Kenny (1939) to determine whether the value which deviated most from the others should be rejected or included in the average. Of the calcium determinations, one rejection was made for each of four samples of greens and of the phosphorus, five rejections.

RESULTS AND DISCUSSION

A summary of the record at harvesting and of chemical analysis is given (Table 1), the results of statistical analysis of the data which was made to determine whether method of sampling influenced the content of calcium, phosphorus, and iron are presented (Tables 2 and 3).

TABLE 2

Variance Analysis of Total-Population Samples

Source of variance	d f	Ca		P		Fe	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Total	15	.3941	.02627	.011955	.0007970	1373.718	91.5812
Between rows	3	.1153	.03843	.007768	.0025893[1]	1113.073	371.0243[1]
Within rows	12	.2788	.02323	.004187	.0003489	260.645	21.7204
		F = 1.65		F = 7.42		F = 17.08	

[1] Highly significant.

Two approaches were employed in the statistical treatment—analysis of variance of the total-population samples and test for significance of difference between two means applied both to the random and the total-population samples. In each of the two sets of random samples the mean value of the four single rows was compared with the mean of the corresponding four composite samples. With the total-population samples, the values for the four plats in each row were averaged for each mineral to give a mean value for the row, and every possible comparison was made between rows.

The variance analysis (Table 2) shows that respecting calcium the variation between rows was not significantly greater than within rows; but respecting both phosphorus and iron, variation between rows was significantly greater at the one per cent level than within rows. Similarly (Table 3) no significant differences were found in calcium content in any

[4] Eating quality and some aspects of composition of turnip greens at successive stages of growth. Food Research 9, 42-55.

[5] Grateful acknowledgment is made to the Mississippi Agricultural Experiment Station for generosity in permitting analyses in connection with this study to be made in its Home Economics Laboratory.

TABLE 3

Results of Statistical Analysis

Row numbers		Description of sample	Number of samples	Moisture-free basis								
				Ca (pct.)			P (pct.)			Fe (p.p.m.)		
				Mean ± S E	Comparison	t value	Mean ± S E	Comparison	t value	Mean ± S E	Comparison	t value
2, 3, 4, 5	Random	Single row	4	2.85±.105	Row *vs.* composite	0.409	.726±.0213	Row *vs.* composite	0.144	155±6.35	Row *vs.* composite	0.109
2, 3, 4, 5	Random	Composite	4	2.80±.063			.730±.0087			154±3.71		
16, 17, 18, 19	Random	Single row	4	3.11±.027	Row *vs.* composite	7.211[1]	.650±.0209	Row *vs.* composite	1.776	135±5.19	Row *vs.* composite	1.145
16, 17, 18, 19	Random	Composite	4	2.69±.052			.692±.0107			144±6.47		
2			4	2.87±.063	Row 2 *vs.* 3	1.850	.631±.0108	Row 2 *vs.* 3	0.835	125±1.91	Row 2 *vs.* 3	10.718[1]
3		All plants	4	2.69±.074	Row 2 *vs.* 4	2.871	.641±.0051	Row 2 *vs.* 4	3.455[2]	103±0.85	Row 2 *vs.* 4	4.590[2]
4			4	2.65±.044	Row 2 *vs.* 5	0.637	.685±.0112	Row 2 *vs.* 5	0.064	110±2.60	Row 2 *vs.* 5	1.994
5		in each plat	4	2.79±.109	Row 3 *vs.* 4	0.464	.632±.0090	Row 3 *vs.* 4	3.557[2]	118±3.24	Row 3 *vs.* 4	2.777
					Row 3 *vs.* 5	0.759		Row 3 *vs.* 5	0.877		Row 3 *vs.* 5	4.445[2]
					Row 4 *vs.* 5	1.193		Row 4 *vs.* 5	3.681[2]		Row 4 *vs.* 5	1.756

[1] Highly significant. [2] Significant.

row *vs.* row comparison of the total-population samples; but there are some significant differences respecting phosphorus and iron.

Row 4 with the highest content of phosphorus (0.685 per cent) among the total-population samples differs significantly from the other three rows; but no two of the other three rows, with phosphorus contents of 0.631, 0.641, and 0.632 per cent, differ significantly. In iron content, total-population Row 5 (118 p.p.m.) is significantly higher than Row 3 (103 p.p.m.). Difference between Row 2 (125 p.p.m.) and Row 4 (110 p.p.m.) is significant, and between Row 2 and Row 3 highly significant. The remaining differences between rows are not significant.

Among the six comparisons of random samples between single-row and composite samples, two each for calcium, phosphorus, and iron, the t value of the critical ratio was great enough to show statistically significant difference in only one case, the calcium in one pair of mean values of single-row and composite samples.

Explanation of this one significant difference in calcium content between comparable samples taken by different methods is thought to be in the difference in proportion of the original samples which was retained for chemical analysis (Table 1). Although the same person did all the preparation of samples for washing and drying, and made conscious effort to be consistent in the practice of discarding leaves, the difference in mean percentage of good leaves is 9.15 between single-row and composite samples. Mean percentages of good leaves in the other pair of single-row and composite samples differ by only 0.92, and extreme difference among the means of rows in the total-population samples is 3.82. In the pair of means showing a significant difference in calcium content, the sample having the higher calcium content (3.10 *vs.* 2.69 per cent) has also a higher percentage of good leaves, and thus relatively more of the outer leaves and of larger stems.

That stems from these greens were probably higher in calcium than were the leaves is indicated by analysis of samples harvested at intervals from November 4 to 11, inclusive, from the guard rows five feet in length at the end of each of the center 10 rows of the planting. Six such samples separated into stems and leaves were analyzed by the same person who made the determinations of minerals in this study. In all pairs of separated samples, stems were found to contain a higher percentage of calcium than did the leaves, by amounts ranging from 0.17 to 0.62, the average difference being 0.35. Supporting evidence is also afforded by the difference in stem and leaf portions of 10 samples of turnip greens grown in Norfolk, Virginia, in 1938 and analyzed in the Mississippi Experiment Station.[6] In every pair of these 10 samples the percentage of calcium in the stems exceeded that in the leaves by amounts ranging from 0.39 to 1.02, and averaging 0.73.

Although no data are available on the calcium content of inner and outer leaves of turnip greens, there is presumptive evidence in the findings for other leafy vegetables that the outer leaves of turnips may also be higher in calcium content. Cowell (1932) reports that outer leaves of

[6] Private communication from Miss Olive Sheets.

cabbage had 20 to 30 times the calcium content found in the inner leaves. Hsu and Adolph (1935) found two to 20 times as much calcium in the outer leaves as in the inner ones of 10 Chinese vegetables, including rape, spinach, leeks, and cabbage. Wang (1936), including rape, cabbage, spinach, and celery among 11 Chinese vegetables studied, found calcium higher in the outer leaves.

It appears likely then that the statistically significant difference in the mean calcium content of one pair of single-row and composite-random samples was not due to the method of sampling in the field but may have been due to an unintentional variation in preparation of the sample. Such a source of variation, unless fully controlled, might easily be the responsible factor for differences in mineral content attributed to other causes, such as season, fertilizer treatment, type of soil, or variety.

The data indicate for phosphorus and iron content of the total-population samples, that the significant differences found are not due to laboratory technique. The range in mean percentage of good leaves in the four rows is narrow (49.94 to 53.76), moisture content of fresh leaves varies still less (90.90 to 91.53 per cent), and duplicate analyses for iron and triplicate for phosphorus are in close agreement with small standard errors of mean and standard deviations. There are no consistent tendencies between rows in the significant differences found for phosphorus and iron. Row 4 alone varies significantly from every other row in content of phosphorus. Whereas Row 4 is highest in phosphorus content, it holds third rank among the four rows in content of iron. Both Row 2 and Row 5 contained significantly more iron than Row 3, and Row 2 more than Row 4. These differences in iron and phosphorus are considered to be the result of real differences between the plants representing the rows involved. Its probable cause will be taken up later in the discussion.

That four samples was a sufficient number in each set of the mode-of-sampling series is indicated by comparison of standard deviations and coefficients of variability with corresponding constants of the means of the six sets of 10 center rows [7] of greens grown the same season in three localities of Texas—College Station, Winter Haven, and Iowa Park. The two sets of data presented (Table 4) are in remarkable agreement, with exception that coefficients of variability for phosphorus and iron in greens from Iowa Park are two to three times as great as for calcium in the same greens, whereas there are no such wide differences in the method-of-sampling series.

Standard deviations for calcium in the method-of-sampling samples range from 0.054 to 0.218; among center rows from 0.104 to 0.238; similarly for coefficients of variability, from 1.75 to 7.80 compared with a range of 3.97 to 8.32. Respecting phosphorus, among eight standard deviations in the method-of-sampling series, only two are greater than the

[7] Acknowledgment is gratefully made to Dr. G. S. Fraps, Chief, Division of Chemistry, Texas Agricultural Experiment Station, from whose laboratory the data on center-row samples have been made available; and to Mr. L. R. Hawthorn and Dr. B. S. Pickett, who grew the greens at Substation No. 19 and No. 16, respectively, of the Texas Agricultural Experiment Station.

TABLE 4

Standard Deviations and Coefficients of Variability in Mean Content of Calcium, Phosphorus, and Iron in Turnip Greens

(Greens from 1938 crop)

Mineral	Mode of sampling (mean of 4 samples)									Center rows, total population from 10 ft. of each row (mean of 10 samples)				
	Single row, random			Composite random			Total population from 4 ft. of each row			Place grown [1]	Fertilized		Not fertilized	
	Rows	S.D.[2]	C.V.[2]	Rows	S.D.	C.V.	Row No.	S.D.	C.V.		S.D.	C.V.	S.D.	C.V.
Ca	2, 3, 4, 5	0.210	7.38	2, 3, 4, 5	0.125	4.46	2	0.126	4.38	C. S.	0.144	4.74	0.201	6.05
							3	0.149	5.53	W. H.	0.104	3.97	0.126	4.58
	16, 17, 18, 19	0.054	1.75	16, 17, 18, 19	0.103	3.83	4	0.088	3.31	I. P.$_1$	.....	.....	0.190	6.69
							5	0.218	7.80	I. P.$_2$	.....	.....	0.238	8.32
P	2, 3, 4, 5	0.0425	5.86	2, 3, 4, 5	0.0174	2.39	2	0.0216	3.43	C. S.	0.0657	9.82	0.0544	9.07
							3	0.0103	1.60	W. H.	0.0622	9.80	0.0274	4.65
	16, 17, 18, 19	0.0418	6.43	16, 17, 18, 19	0.0213	3.08	4	0.0223	3.26	I. P.$_1$	.....	.....	0.0493	13.58
							5	0.0180	2.85	I. P.$_2$	.....	.....	0.0577	17.59
Fe	2, 3, 4, 5	12.71	8.21	2, 3, 4, 5	7.42	4.82	2	3.81	3.05	C. S.	20.83	13.44	10.45	6.30
							3	1.71	1.67	W. H.	14.44	11.83	11.02	9.00
	16, 17, 18, 19	10.38	7.70	16, 17, 18, 19	12.93	8.96	4	5.20	4.72	I. P.$_1$	.....	.....	31.01	16.52
							5	6.48	5.52	I. P.$_2$	.....	.....	34.08	17.79

[1] C.S. means College Station; W.H. means Winter Haven; I.P. means Iowa Park. [2] S.D. means standard deviation; C.V. means coefficient of variability.

smallest standard deviation among center-row samples (0.0425 and 0.0418 *vs.* 0.0274) and similarly with coefficients of variability (5.86 and 6.43 *vs.* 4.65). With iron, only two of eight standard deviations (12.71 and 12.93) and three coefficients of variability (8.21, 7.70, and 8.96) exceed the two lowest standard deviations and the smallest coefficient of variability, respectively (S.D. 10.45 and 11.02, C.V. 6.30), of center-row values.

To account for the significant differences found in the phosphorus and iron contents of some of the total-population samples, the suggestion is offered that soil differences had more effect upon phosphorus and iron contents than upon calcium, for which no significant variation was found in these same samples. The soil is known to be spotted in character and an old "dead furrow" passed through the corner of planting containing the guard rows from which the total-population samples came. That portion of the planting exhibited the most uneven growth. The figures for yield (Table 1) are evidence of uneven growth. Association of uneven growth with greater variability in content of phosphorus and iron than in calcium was found with the turnip greens grown at Iowa Park. There, two unfertilized plantings of 10 rows each yielded from 10 feet of each of the rows, amounts ranging from 895 to 2,685 grams in one planting and from 520 to 2,534 grams in the other. Coefficients of variability were 13.58 and 17.59 for phosphorus, and for iron 16.52 and 17.79, compared with 6.69 and 8.32 for calcium in the same greens. While the samples in this study of method of sampling do not show commensurate differences in coefficients of variability as between phosphorus and iron *vs.* calcium, it may nevertheless be that variation in character of the soil was an important factor in producing the differences in phosphorus and iron contents. Unfortunately soil analyses were not made at the time nor could that be done later. Hence the relationship suggested between soil character and variation in phosphorus and iron contents of the greens can be only a speculation.

In view of the foregoing observations, the authors are of the opinion that the significant but inconsistent differences found in the phosphorus and iron contents of the total-population samples is not due to the technique involved in sampling.

To determine the approximate content of calcium, phosphorus, and iron in turnip greens, the two randomizing procedures appear equally satisfactory where no more than four rows of 10 to 16 feet are to be sampled. However, if a larger number of rows are included in the experiment, four composite-random samples of 1,500 to 2,000 grams composed of equal portions from the several rows obviously would be more economical than random samples from each row separately, and in all likelihood the four composite-random samples would be representative. On the basis of standard error of the mean and standard deviation, total-population samples from four-foot plats were as good as those from lengths of 10 feet to use as material for mineral analysis (Table 4). Assuming comparable yield per foot, the four-foot length would be the more economical in time and labor to prepare the material for analysis. The shorter length plat as source of a total-population sample would not be a factor of economy,

however, if more than four rows are to be sampled. Composite-random samples would then be the better choice.

SUMMARY AND CONCLUSIONS

Three methods of sampling have been used for harvesting turnip greens —random from single 16-foot rows, composite-random from the same rows, and total-population from 16-foot rows each divided into four plats. Samples consisted of 1,500 to 2,000 grams each. Two sets of four rows each provided the random samples; from each set, four single-row and four composite were taken, a total of 16 samples. For the total-population samples four rows of four plats each were used to supply 16 samples. Moisture in the fresh greens and calcium, phosphorus, and iron in the dried material were determined.

Mean values of four samples from each method of sampling gave acceptably low standard errors and standard deviations in comparison with total population from 10-foot rows. With calcium only one significant difference was found between comparable samples, which were one pair of single-row and composite-random samples. The explanation offered is the higher proportion of outer leaves in the single-row sample, which was higher in calcium, than in the composite. Significant differences in phosphorus and iron contents were found in only part of the total-population samples. One row was significantly higher in phosphorus than the other three which did not differ significantly among themselves. Three significant differences were found among six row *vs.* row comparisons of iron in total-population samples. All rows were involved in these differences. The rows ranked in an entirely different order in mean content of iron than in phosphorus; the row of highest value in phosphorus held third place in iron. The possibility is suggested that variation in soil was responsible for the inconsistent differences in phosphorus and iron.

The findings in this study point to the economy of time and labor in the use of no more than four random-composite samples taken from several rows to determine the approximate composition of greens, provided experimental design, sufficient homogeneity of the soil, and uniformity of cultural treatment permit.

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EFFECT OF FREEZING ON UTILIZATION OF CANNED FOODS

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To obtain information on changes that may occur in canned foods when they are frozen through exposure to severe winter temperatures, the writers conducted a series of tests on 35 canned products packed in tin containers. All cans were of normal commercial fill and so selected as to represent a broad range of differences in physical form and texture that might be affected by freezing. The work was designed to include and facilitate observations on the following points:

1. Appearance of the frozen cans.
2. Possible loss of vacuum indicating leakage owing to freezing.
3. Change in drained weight.
4. Change in appearance after slow thawing.
5. Change in appearance after quick thawing.
6. Change in appearance after slow thawing and cooking.
7. Change in appearance after quick thawing and cooking.
8. Change in cooking properties.
9. Change in the inside or outside of the container.

FOODS INCLUDED IN STUDY

Vegetables

Asparagus
Wax beans
Lima beans
Beets
Carrots
Whole grain corn
Cream style corn
Whole grain vacuum pack corn
Alaska peas
Sweet peas
Pumpkin
Spinach
Sweet potatoes
Tomatoes

Fruits

Boysenberries
Red tart pitted cherries
Fruit cocktail
Peaches
Pears
Pineapple
Fresh prunes (plums)

Miscellaneous

Salmon
Sardines, Maine
Sardines (pilchards)
Shrimp
Herring roe
Potted meat
Army Ration M1, beans and meat
Army Ration M2, corn beef hash
Army Ration M3, vegetable stew
Milk
Strained spinach
Tomato soup
Vegetable soup
Clam chowder

EXPERIMENTAL PROCEDURE

Nine cans from a single lot of each product were obtained and stored at about 29°C.(85°F.) overnight. The "flip vacuum" of each can was determined and recorded. Three cans of each were held at room temperature for controls. Six cans of each product were frozen solid by placing them in a refrigeration room held at about —18°C.(0°F.) for seven days. Four frozen cans of each product were then taken out of the freezer and allowed to thaw at room temperature. Two of these thawed cans were returned to the freezer and left for an additional seven-day period.

In order to make critical comparisons, all of the nine cans of each product were examined at the same time. The two cans that had been frozen twice were removed from the freezer 24 hours before the test was to be made and allowed to thaw. The "flip vacuum" at 85°F. of the two cans frozen once and the two cans frozen twice was then determined. (See page 74 for explanation of this test.) The two remaining frozen cans were taken direct from the refrigeration room for examination.

The external condition of the frozen cans was observed and compared with the control cans and those that had been frozen and thawed.

In the examination of the contents each of the three control cans, the two cans frozen once, and the two cans frozen twice were emptied into separate pans. Texture, color, separation of liquid, flavor, and general appearance were noted. In each case the contents of the cans that had been frozen were compared directly with the control cans. On those products where freezing might cause change in the proportion of liquids and solids the drained weight was determined by draining the contents on an eight-inch screen for two minutes in the usual way. The volume of drained solid and of liquid was measured separately in standard measuring cups.

The cans that were taken directly from the freezer and brought for test examination were treated in several ways in order to suggest means for using cans that were frozen solid. Cans were allowed to thaw at room temperature for a short time, immersed in hot water for varying times, etc.

Those foods which are usually served hot were heated in a saucepan. The method used was kept as simple as possible to hold to a minimum the effect of factors other than freezing. Vegetables, soups, and strained foods were emptied into saucepans (no seasonings were added) and heated to boiling. The heat was then reduced and the food simmered two minutes to heat through the solid portion.

Evaporated milk was used in coffee and cocoa and also made into a cream sauce. Red tart pitted cherries and pumpkin were made into pies.

Fish, sea food, and all of the fruits, except red tart pitted cherries, were examined and sampled as they came from the can without being heated.

The cans were rinsed and examined both externally and internally for possible changes brought about by freezing.

RESULTS OBTAINED

The analytical data obtained in the examination of the samples are presented (Table 1); in this table the terms "very slight," "slight," and "noticeable" are used to indicate varying degrees of change. As so used, "very slight" means detectable only by direct comparison with the control cans; "slight" means perceptible to a close observer; "noticeable" means easily perceptible variation from normal condition.

Most cans containing foods that have frozen will have both ends slightly bulged. This is due to the expansion of the contents on freezing. On thawing, the ends return to their normal position. Cans containing foods such as salmon or vacuum-packed whole kernel corn, which contain little free liquid, will usually not have either end bulged on freezing.

TABLE 1

Results of Freezing Treatments—Detailed Record of Observations

Product	Can size	Treatment	"Flip vacuum"		Drained weight	Contents		Effects of freezing
			Original	Final		Liquid	Solid	
			in.	*in.*	*oz.*	*cups*	*cups*	
Asparagus, all-green stalks	2	None (control)	3.5	....	14.5	3/4	.....	Stalk structure weakened. Liquid drained away leaving soft fibrous pieces having little flavor. Flavor of liquid not changed. Color of liquid and stalks not changed. On heating the same changes were still noticeable.
		None (control)	12.5	....	11.6	1 1/16	.....	
		None (control)	0	....	13.4	7/8	.....	
		Frozen once	0	0	11.1	1 3/16	.....	
		Frozen once	9.5	9.5	11.6	1 1/8	.....	
		Frozen twice	8.5	9	11.5	1 1/8	.....	
		Frozen twice	6.5	7.5	10.1	1 5/8	.....	
Beans, wax, cut, stringless	2	None (control)	26	....	13.1	7/8	2 1/2	Slight softening of the structure of the pods of the beans, more noticeable when beans were heated. Some mashing of the beans on stirring. No difference in color or flavor. Liquid not changed.
		None (control)	28	....	12.2	1	2 1/2	
		None (control)	24	....	12.2	1	2 1/2	
		Frozen once	26	26	11.6	1	2 1/4	
		Frozen once	25	26	12.2	1	2 1/2	
		Frozen twice	22	22	12.2	1	2 1/4	
		Frozen twice	22	24	11.6	1	2 1/3	
Beans, lima, green and white	1	None (control)	25	....	7.8	1/8	1 1/2	No difference in texture, appearance, or flavor of beans or liquid either cold or after heating.
		None (control)	30	....	8.1	3/8	1 1/3	
		None (control)	28	....	.....		.....	
		Frozen once	24	24	7.9	3/8	1 1/3	
		Frozen once	25	23	8.6	1/4	1 1/2	
		Frozen twice	25	24	8.5	3/8	1 7/16	
		Frozen twice	22	22	8.3	1/3	1 7/16	
Beets, diced	2	None (control)	26	....	14.6	3/4	2 1/2	No difference in texture, color, or flavor.
		None (control)	25	....	14.1	3/4	2 1/2	
		Frozen once	24	25	14.3	1 3/16	2 1/2	
		Frozen once	15	15	14.3	3/4	2 1/2	
		Frozen twice	24	25	14.5	3/4	2 1/2	
Carrots, diced	2	None (control)	....	....	13.7	7/8	2 1/2	Frozen twice, slightly softer in texture but not broken. No difference in color or flavor.
		None (control)	....	....	14.2	1 3/16	2 2/3	
		Frozen once	....	....	14.2	1 3/16	2 1/2	
		Frozen once	....	....	14.2	7/8	2 1/2	
		Frozen twice	....	....	13.2	7/8	2 1/3	
Corn, whole grain Bantam	2	None (control)	30	....	15.2	7/16	2 2/3	Very slight softening of the inner portion of the kernels. No difference in color or flavor either cold or after heating.
		None (control)	30	....	15.0	3/4	2 2/3	
		None (control)	30	....	15.2	10/16	2 1/2	
		Frozen once	30	30	14.7	3/4	2 1/2	
		Frozen once	30	30	14.8	3/4	2 1/2	
		Frozen twice	30	30	14.5	3/4	2 1/3	
		Frozen twice	30	30	14.3	3/4	2 1/3	

		Frozen once	30	30	14.8	¾	2½	ing.
		Frozen twice	30	30	14.5	¾	2⅓	
		Frozen twice	30	30	14.3	¾	2⅓	

TABLE 1 *(Continued)*

Corn, cream style	2	None (control)	30	....	.....	.....	2¼	Noticeable difference in physical condition of the cream portion, which was no longer creamy but spongy and granular in texture, slightly thickened, and from which a clear liquid slowly separated. On heating, this condition disappeared and the corn was normal in every way. No difference in color or flavor.
		None (control)	30	....	.....	.....	2¼	
		None (control)	30	....	.....	.....	2⅓	
		Frozen once	30	30	.....	.....	2⅓	
		Frozen once	30	30	.....	.....	2¼	
		Frozen twice	30	30	.....	.....	2⅓	
		Frozen twice	30	30	.....	.....	2⅓	
Corn, vacuum pack	2	None (control)	30	....	.....	.....	1¾	No difference in separation of liquid or color, texture, or flavor.
		None (control)	27	....	.....	.....	1¾	
		None (control)	27	....	.....	.....	1¾	
		Frozen once	27	25	.....	.....	1¾	
		Frozen once	27	25	.....	.....	1¾	
		Frozen twice	27	25	.....	.....	1 13/16	
		Frozen twice	27	26	.....	.....	1¾	
Peas, Alaska, No. 2 size, very young	2	None (control)	25	....	12.9	⅞	2⅛	No difference in texture, color, or flavor, either cold or hot. Liquor clear.
		None (control)	23	....	12.9	1	2⅛	
		None (control)	23	....	12.9	1	2⅓	
		Frozen once	24	23	12.9	1	2⅓	
		Frozen once	25	24	12.9	1	2⅓	
		Frozen twice	25	27	12.9	1	2⅓	
		Frozen twice	24	23	12.9	1	2⅓	
Peas, sweet, small, very young	2	None (control)	21	....	13.4	⅞	2⅛	Very slight softening of the peas. No breakage. Flavor and color not changed. Same difference in texture after heating.
		None (control)	21	....	13.4	⅞	2⅓	
		None (control)	19	....	13.8	⅞	2⅓	
		Frozen once	23	24	13.3	1	2 1/16	
		Frozen once	21	21	13.4	⅞	2¼	
		Frozen twice	19	19	13.4	1	2 1/16	
		Frozen twice	20	21	13.4	15/16	2	
Pumpkin	2	None (control)	23	....	.....	.....	.....	Noticeably thinner consistency, with somewhat more separation of liquid on standing. No difference in color or flavor. When baked in pies no difference in texture, color, or flavor.
		None (control)	24	....	.....	.....	.....	
		Frozen once	25	22	.....	.....	.....	
		Frozen once	17	17	.....	.....	.....	
		Frozen twice	27	23	.....	.....	.....	

TABLE 1 *(Continued)*

Product	Can size	Treatment	"Flip vacuum"		Drained weight	Contents		Effects of freezing
			Original	Final		Liquid	Solid	
			in.	*in.*	*oz.*	*cups*	*cups*	
Spinach	2	None (control)	18	....	13.5	9/16	2	Slight softening of the structure of the leaves but not mushy, and leafy character still present. Same slight difference after heating. No change in color or flavor.
		None (control)	17	....	13.2	3/4	1 3/4	
		None (control)	18	....	13.2	2/3	2	
		Frozen once	19	19	11.0	1 5/16	1 5/16	
		Frozen once	16	16	12.0	1 3/16	1 2/3	
		Frozen twice	15	15	11.7	7/8	1 3/4	
		Frozen twice	17	17	11.7	7/8	1 2/3	
Sweet potatoes	2	None (control)	10	....	.....	.....	.....	No separation of liquid. No difference in texture, color, or flavor.
		None (control)	14	....	.....	.....	.....	
		Frozen once	10	10	.....	.....	.....	
		Frozen once	5	....	.....	.....	.....	
		Frozen twice	4	2	.....	.....	.....	
Tomatoes	2	None (control)	16	....	12.5	1 1/2	3/4	Slight softening of the flesh of tomatoes. No difference in color or flavor.
		None (control)	14	....	10.1	1 3/16	1 1/8	
		None (control)	13	....	10.6	1 1/4	1 1/8	
		Frozen once	15	15	7.8	1	1 1/3	
		Frozen once	15	16	8.1	1	1 1/4	
		Frozen twice	15	15	9.6	1 1/16	1 3/16	
		Frozen twice	15	16	7.7	7/8	1 1/3	
Boysenberries	2	None (control)	20	....	10.6	1 1/2	1 1/4	No difference in texture, color, or flavor.
		None (control)	19	....	11.0	1 1/8	1 1/3	
		None (control)	22	....	11.6	1 1/3	1 5/16	
		Frozen once	24	23	11.5	1 1/4	1 5/16	
		Frozen once	20	13	10.1	1 1/4	1 3/16	
		Frozen twice	17	16	10.7	1 1/3	1 1/4	
		Frozen twice	21	21	10.6	1 1/4	1 1/4	
Cherries, red pitted	2	None (control)	26	....	12.7	7/8	2 1/4	No difference in color, flavor, or texture. Made into pies, no difference could be detected.
		None (control)	24	....	13.4	13/16	2 1/3	
		None (control)	20	....	14.1	3/4	2 1/3	
		Frozen once	24	24	13.8	9/16	2 1/4	
		Frozen once	30	29	13.0	13/16	2 1/4	
		Frozen twice	22	22	12.3	1	2	
		Frozen twice	25	25	13.0	7/8	2 1/4	
Fruit cocktail	8 oz.	None (control)	24	....	5.3	.....	.....	No difference in color, flavor, or texture of any part of the product.
		None (control)	23	....	5.1	.....	.....	
		Frozen once	30	30	5.7	.....	.....	
		Frozen once	21	21	5.5	.....	.....	
		Frozen twice	20	20	5.3	.....	.....	
		Frozen twice	26	25	5.5	.....	.....	

		Frozen twice	20	20	5.3	.....	.....	
		Frozen twice	26	25	5.5	.....	.....	

TABLE 1 *(Continued)*

Peaches, halves, clingstone	1	None (control)	....	....	8.8	3/4	.....	No difference in texture, color, or flavor.
		None (control)	17	....	9.0	13/16	.....	
		None (control)	25.5	....	9.2	13/16	.....	
		Frozen once	16	16	9.0	13/16	.....	
		Frozen once	20	16	10.2	5/8	.....	
		Frozen twice	23	23	8.7	3/4	.....	
		Frozen twice	25	24	9.0	3/4	.....	
Pears, halves, Bartlett	2½	None (control)	19	....	20.3	13/16	.....	Very slight weakening of the structure. No difference in color or flavor.
		None (control)	20	....	19.2	1¼	.....	
		None (control)	....	....	19.6	1¼	.....	
		Frozen once	20	19	18.5	15/16	.....	
		Frozen once	19	18	19.4	1¼	.....	
		Frozen twice	21	21	16.6	1⅝	.....	
		Frozen twice	19	19	17.3	1½	.....	
Pineapple slices	9 oz.	None (control)	24	....	5.8	7/16	.....	No difference in texture, color, or flavor.
		None (control)	23	....	5.8	7/16	.....	
		None (control)	22	....	5.8	7/16	.....	
		Frozen once	23	22	6.1	7/16	.....	
		Frozen once	24	21	5.7	½	.....	
		Frozen twice	23	23	5.6	½	.....	
		Frozen twice	22	22	6.0	7/16	.....	
Plums, whole	2½	None (control)	16	....	16.2	1⅝	.....	No difference in texture, color, or flavor.
		None (control)	....	....	14.8	1¾	.....	
		None (control)	30	....	16.6	1¾	.....	
		Frozen once	13	11	16.9	1⅞	.....	
		Frozen once	30	30	16.0	1¾	.....	
		Frozen twice	24	18	14.1	1⅞	.....	
		Frozen twice	25	25	15.9	1¾	.....	
Salmon	½ lb. flat	None (control)	21	....	.....	.....	.....	No difference in texture, color, or flavor.
		None (control)	29	....	.....	.....	.....	
		None (control)	26	....	.....	.....	.....	
		Frozen once	26	26	.....	.....	.....	
		Frozen once	29	28	.....	.....	.....	
		Frozen twice	26	26	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
Sardines, Maine in oil	¼ oil	.....	No measurements made		.....	.....	.....	No difference in texture, color, or flavor.
Sardines, California pilchards in tomato sauce	1 lb. oval	.....	No measurements made		.....	.....	.....	No difference in texture, color, or flavor.

TABLE 1 *(Continued)*

Product	Can size	Treatment	"Flip vacuum"		Drained weight	Contents		Effects of freezing
			Original	Final		Liquid	Solid	
			in.	*in.*	*oz.*	*cups*	*cups*	
Shrimp, brine pack	1	None (control)	25	....	6.7	5/16	1 9/16	No difference in texture, color, or flavor.
		None (control)	30	....	7.4	1/2	1 1/2	
		None (control)	30	....	6.9	5/8	1 1/2	
		Frozen once	30	23	6.9	1/2	1 1/2	
		Frozen once	30	30	7.4	1/2	1 9/16	
		Frozen twice	30	27	7.4	1/2	1 9/16	
		Frozen twice	30	28	7.4	1/2	1 9/16	
Herring roe	8 oz.	None (control)	9	....	5.7	.....	.....	No difference in texture, color, or flavor.
		None (control)	14	....	5.8	.....	.....	
		None (control)	15	....	5.3	.....	.....	
		Frozen once	8	9	6.0	.....	.....	
		Frozen once	15	15	6.2	.....	.....	
		Frozen twice	11	9	5.8	.....	.....	
		Frozen twice	12	11	5.3	.....	.....	
Milk, evaporated	Small	.....	No measurements made		.....	.....	.....	Some thickening noticeable when poured out at room temperature. Not curdled or lumpy. No separation of fat. No difference in color or flavor. No difference when used for cream sauce, coffee, or cocoa.
Potted meat	4 oz.	None (control)	20	....	.....	.....	.....	No difference in texture, color, or flavor.
		None (control)	23	....	.....	.....	.....	
		None (control)	30	....	.....	.....	.....	
		Frozen once	30	30	.....	.....	.....	
		Frozen once	30	30	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
Soup, tomato, condensed	1	None (control)	30	....	.....	.....	1 1/3	Noticeable difference in appearance and texture. The cooked starch was spongy and granular and liquid drained away on standing. No difference in color or flavor. When heated with an equal amount of water for serving, the abnormal appearance and texture entirely disappeared.
		None (control)	30	....	.....	.....	1 1/3	
		None (control)	30	....	.....	.....	1 1/3	
		Frozen once	21	21	.....	.....	1 1/3	
		Frozen once	30	30	.....	.....	1 1/3	
		Frozen twice	30	30	.....	.....	1 1/3	
		Frozen twice	30	30	.....	.....	1 1/3	

								normal appearance and texture entirely disappeared.

TABLE 1 *(Concluded)*

Soup, vegetable, condensed	1	None (control)	15	....	.....	.....	1⅓	Difference in appearance and texture of the cream portion. Same as tomato soup.
		None (control)	30	....	.....	.....	1⅓	
		None (control)	16	....	.....	.....	1⅓	
		Frozen once	15	16	.....	.....	1⅓	
		Frozen once	13	14	.....	.....	1⅓	
		Frozen twice	17	17	.....	.....	1⅓	
		Frozen twice	30	23	.....	.....	1⅓	
Strained food: spinach	Small	None (control)	30	....	.....	.....	.....	Very slight softening of the tissues and a slight separation of a clear liquid. No difference in color or flavor. Same difference when heated.
		None (control)	30	....	.....	.....	.....	
		None (control)	30	....	.....	.....	.....	
		Frozen once	30	30	.....	.....	.....	
		Frozen once	30	30	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
Soup, clam chowder, condensed	1	None (control)	30	....	.....	.....	1⅓	Difference in appearance and texture of the cream portion —same as tomato soup.
		None (control)	18	....	.....	.....	1⅓	
		Frozen once	30	15	.....	.....	1⅓	
		Frozen once	30	15	.....	.....	1⅓	
		Frozen twice	30	19	.....	.....	1⅓	
		Frozen twice	30	11	.....	.....	1⅓	
Army Ration M1 (beans and meat)	1 lb.	None (control)	....	....	.....	.....	.....	No difference in texture, color, or flavor of the beans or meat.
		None (control)	3	....	.....	.....	.....	
		Frozen once	0	0	.....	.....	.....	
		Frozen once	2	2	.....	.....	.....	
		Frozen twice	30	30	.....	.....	.....	
Army Ration M2 (corn beef hash)	.....	None (control)	10	....	.....	.....	.....	Very noticeable difference in texture—dry, granular. Slight separation of liquid. No difference in color. Normal texture entirely restored by warming and stirring in a pan.
		None (control)	30	....	.....	.....	.....	
		Frozen once	13	13	.....	.....	.....	
		Frozen once	12	12	.....	.....	.....	
		Frozen twice	12	11	.....	.....	.....	
Army Ration M3 (vegetable stew)	.....	None (control)	7	....	.....	.....	.....	No difference in meat. Slight softening of the carrots. Distinct change in texture of the potatoes, which were dry and granular. On heating the normal texture of the potatoes was restored.
		None (control)	0	....	.....	.....	.....	
		Frozen once	10	9	.....	.....	.....	
		Frozen once	2	2	.....	.....	.....	
		Frozen twice	9	9	.....	.....	.....	

None of the cans examined showed evidence of leakage or serious straining of the seams. The vacuum after freezing and thawing is practically the same as before freezing.

The "flip vacuum" test is a method of measuring at different times relative changes in the degree of vacuum within the can. A special plate with rubber gasket and a valve attachment is used to produce any desired degree of vacuum outside of one end of the can. When this vacuum outside overcomes the vacuum inside plus the rigidity of the metal in that can end, the end snaps from the concave to the convex position and this "flip" is used as an indication of the degree of vacuum in the can. Change in the gas pressure inside the can is revealed by any change in the "flip vacuum" results. The inches of vacuum indicated by this test will be higher than the actual vacuum within the can because of the rigidity of the metal in the can end.

Certain products showed some loss of drained weight brought about by freezing. This is apparently due to a weakening of the structure of certain parts of the plant. The most conspicuous example was canned asparagus, the structure of the stalks of which was weakened to such an extent that the liquid drained away from the stalks and left a soft fibrous mass with a shrunken appearance. The drained weight of the frozen sample was considerably less than that of the control sample. Less noticeable was the change brought about in canned tomatoes and canned wax beans.

This weakening of the structure of vegetable fibers brought about by freezing has been observed previously by different research workers connected with the frozen-food industry. A book by Tressler and Evers, "The Freezing Preservation of Fruits, Fruit Juices, and Vegetables" (Avi Publishing Co., New York, N. Y.), gives on page 75 an excellent summary of these changes, which are said to be due to the breaking down of the contents of the cells which formerly gave support to their walls. A noticeable amount of cellular breakdown in asparagus that has been frozen is discussed and pictured in an article by Cox and McMasters, "Microscopic Studies of Tissue of Frozen Fruits and Vegetables" (*Food Research* 7, 135-139).

Attempts to use solidly frozen cans of food were not successful. It was very difficult to open the cans and the contents could not be removed while frozen.

Immersing the frozen can in a pan of hot water for 15 minutes thawed the outside of the mass sufficiently to permit opening the can and removing the contents. Allowing the frozen cans to stand at room temperature for two hours brought about a similar partial thawing. Attempts to use partly thawed products without heating resulted in some tearing of the pieces of food. Most products that were served hot could be heated in the usual way. Asparagus and tomatoes broke up on heating; this affected appearance but not flavor. Wherever possible the frozen cans of food should be thawed slowly by letting them stand at room temperature overnight.

The most noticeable change owing to freezing was that produced in canned foods containing large proportions of cooked starch; for example, Army Ration M2 (corned beef hash), cream style corn, and concentrated soups. Freezing changed the physical nature of the cooked starch com-

pletely. Instead of a thick, viscous mass it became spongy and granular, and a clear liquid separated quickly from the mass on standing in a pan. The sensation to the taste was strange and unpleasant. Remarkably, however, the original characteristics of the cooked starch were completely restored when the material that had been frozen was heated in an open pan. As soon as the material was heated through, the original texture was restored. Storage of the frozen and thawed cans for two months at 36.7°C. (98°F.) did not restore the original texture of the cooked starch.

There was no change at all in the appearance of the inside or outside of the containers owing to freezing. (Sweating of the cans and labels when brought from the freezing room has been disregarded in these tests.)

SUMMARY

The effect of freezing on the utilization of a number of canned foods has been studied. Frozen canned foods should be allowed to thaw gradually and used in the usual manner. Canned foods containing cooked starch show a difference in texture due to freezing but this entirely disappears on heating the contents for serving. A few of the products studied show some softening of the tissues. The ends of most solidly frozen cans will be slightly bulged due to expansion of the contents but this bulge disappears when the contents are thawed.

ASCORBIC ACID VALUE OF THE SWEET POTATO AS AFFECTED BY VARIETY, STORAGE, AND COOKING[1]

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The objects of this study have been (1) to determine the significance of the sweet potato as a source of ascorbic acid; (2) to determine the effect of storage upon ascorbic acid value; (3) to determine the ascorbic acid value of one standard variety of sweet potatoes when cooked by accepted methods; and (4) to determine varietal differences in ascorbic acid value so that breeding stock and new varieties can be selected with ascorbic acid value as one of the criteria of the desirability of a variety.

Burrell and Ebright (1940) report a value of 0.326 milligram ascorbic acid per gram for Jersey sweet potatoes, the results based on two samples of potatoes from the market. The season when the determinations were made is not stated. Floyd and Fraps (1939) report an average ascorbic acid value of 0.203 milligram per gram for Porto Rico sweet potatoes with values for three samples which ranged from 0.154 to 0.259 milligram per gram. Again, the season is not stated. Richardson and Mayfield (1941) report a value of 0.211 milligram of ascorbic acid per gram for cooked sweet potatoes. No statement is made concerning the variety. These authors also list sweet potatoes as good sources of vitamin C when considered in relation to cost. Newton and Lowry (1937), using the bioassay method, found that Porto Rico sweet potatoes contained slightly less than 0.2 Sherman unit of vitamin C per gram (corresponding to 0.10 to 0.15 milligram per gram) when fed raw or boiled, and that several months' storage had no appreciable effect on this value. Baked sweet potatoes were found by these authors to be approximately two-thirds as rich in vitamin C content as raw or boiled.

Scoular and Eakle (1943) found that unpeeled Unit I Porto Rico sweet potatoes had ascorbic acid values ranging from 0.032 milligram per gram at the root end to 0.095 milligram per gram at the stem end. These authors report average ascorbic acid values for different varieties which had been stored six months at 10°C.(50°F.), ranging from 0.011 milligram per gram for Unit I Porto Rico to 0.027 milligram per gram for the Old Porto Rico variety.[3] Changes in ascorbic acid value owing to cooking in their study ranged from an average loss of 85 per cent by baking the Nancy Hall variety to an average gain of 345 per cent by boiling the same variety. Candied sweet potatoes showed an average increase of 108 per cent.

The results of all authors except the last named indicate that the sweet potato may be of considerable significance as a source of ascorbic acid in

[1] Approved for publication by the director of the Louisiana Agricultural Experiment Station.

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[3] Recalculated from the author's tables.

southern diets. The study of Scoular and Eakle (1943) suggests the need of more work in regard to the effect of storage conditions and of cooking upon the ascorbic acid value of this vegetable.

EXPERIMENTAL PROCEDURE

During the fall to spring season of 1941-42, the ascorbic acid value of two standard varieties and a number of hybrid varieties of sweet potatoes grown at Louisiana State University Agricultural Experiment Station, Baton Rouge, Louisiana, were determined at approximately two-month intervals to determine varietal differences and the effect of storage. During this season, comparisons were made on the basis of the average of values obtained from sampling each of three to five potatoes, separate determinations being made in duplicate on each potato. It was found that there was a wide variation between individual potatoes of the same variety at the same season; representative figures showing values for individual potatoes are shown (Table 1). Two varieties showed apparent increases in

TABLE 1

Ascorbic Acid Value[1] *of Individual Sweet Potatoes, January, 1942*

Unit I Porto Rico	Triumph
.180	.106
.188	.158
.215	.183
.220	.168
.172	.157
Average .195	.154

[1] Expressed in milligrams per gram.

their ascorbic acid content after two months of storage. It was decided, therefore, to repeat the experiment the following year, sampling 10 potatoes from each variety and, to save time, to make determinations on a composite sample of five potatoes at a time.

In the beginning phase of the work it was found that the proximal end and the distal end of sweet potatoes had very different ascorbic acid values. On one potato a circular slice from the proximal end was found to contain 0.211 milligram per gram, while a similar slice from the distal end was found to contain 0.170 milligram per gram.

The pared portion of two samples was found to have an ascorbic acid value approximately 50 times that of a thin paring from the same raw potato. It was finally decided to use 25-gram samples in the form of a wedge-shaped slice from end to end of each potato. In order to avoid variations owing to difference in the thickness of parings and greater losses in handling owing to exposure of cut surfaces to air, the potatoes were not pared. Samples were placed in a Waring blender and ground for three minutes in a three-per cent metaphosphoric acid solution under carbon dioxide and transferred to a one-liter volumetric flask. Similar samples from four additional potatoes were ground in the same manner and added to the flask. The blender bowl and lid were then rinsed into the flask and the whole made up to volume and thoroughly mixed. A duplicate com-

posite sample was made by taking similar slices from the opposite sides of the same potatoes. Portions from each composite sample were centrifuged and titrated in the usual manner with 2,6-dichlorophenolindophenol solution which was standardized each day against pure ascorbic acid.

In the beginning of this study, hydrogen sulfide regeneration was carried out on each sample to determine the reversibly oxidized ascorbic acid present. A suitable aliquot was buffered to a pH of 3.7 and treated with hydrogen sulfide for 15 minutes, followed by carbon dioxide for three to four hours. The reducing value of these samples was invariably from 10 to 15 per cent greater than that for samples not treated with hydrogen sulfide, even when freshly harvested sweet potatoes, ground under carbon dioxide, were used. Cooked sweet potatoes never showed this increase in reducing value after treatment with hydrogen sulfide. The determination of dehydroascorbic acid was discontinued as it was felt the increase in reducing value after hydrogen sulfide treatment was due to substances other than dehydroascorbic acid. The results on long-stored samples, however probably do not represent the entire antiscorbutic value of the sweet potatoes.

TABLE 2

Ascorbic Acid Value[1] of Sweet Potatoes as Affected by Variety and Storage, 1942-43

Variety	October and November	January	March	May	Loss
					pct.
Unit I Porto Rico	.265	.213	.203	.191	27.9
Nancy Hall	.333	.....	.246	.203	30.0
Triumph	.235	.169	.166	.....	29.4
47442	.303	.....	.209	.189	37.6
1 x 6—39-10[2]	.327	.221	.209	.197	39.8
6 x 1—39-6[2]	.305	.....	.....	.....	.....
1 x 6—39-6[2]	.317	.....	.....	.....	.....

[1] Expressed in milligrams per gram. [2] L.S.U. Experiment Station seedlings derived from crosses between the Porto Rico and Nancy Hall varieties.

Variety and Storage Studies: Beginning October 8, 1942, four standard varieties of sweet potatoes and some seedling varieties were sampled as rapidly as possible at approximately two-month intervals, sampling as described above. The sweet potatoes were stored in a room where temperature varied from 10 to 26.7°C.(50 to 80°F.). This corresponds to common farm practice. The potatoes showed some sprouting at the second period of sampling and increasing amounts with each later sampling but were in good edible condition at the end of the season. All varieties decreased gradually in ascorbic acid value in storage, the greatest losses occurring during the first two-months period. The varieties showing the highest ascorbic acid content lost a higher percentage of their original ascorbic acid content so that, at the end of the storage period, varietal differences became less significant (Table 2).

The Nancy Hall variety and a U. S. Department of Agriculture variety, designated as Introduction No. 47442, gave significantly higher values early in the season. Certain other varieties produced by crossing one of these with the Porto Rico variety gave high values at this time. It would

seem, therefore, that it is feasible to produce new varieties of high ascorbic acid content by selecting parent stock of high value in this respect.

Cooking Studies: Results on cooking losses are based on samples of Unit I Porto Rico sweet potatoes cooked during January and February of 1943. At this season the use of sweet potatoes is at its peak, as indicated by the extent of shipping.

Boiling and Pressure Cooking: In view of the variation in ascorbic acid value of individual potatoes, care was taken to use comparable samples for comparison on the cooked and on the raw basis. When portions of pared potato were boiled, "candied," or cooked in the pressure saucepan, two 25-gram portions of the same end of the same potato were analyzed for ascorbic acid in the raw state. Results of cooking losses were based on the average of four portions cooked separately. In the boiling of pared potatoes each 25-gram sample was cooked separately in a glass beaker using 100 cubic centimeters of distilled water. The weighed sample was dropped into rapidly boiling water, covered with a watch glass, and the flame lowered to a point where the water boiled gently. It was found necessary to cook the potato 20 minutes. This gave a product which held its shape well and was sufficiently soft.

The same method was used for sampling and for cooking "candied" sweet potatoes, except that 25 grams of sugar was dissolved in the cooking water before adding the potato. In each case the cooked potato was dropped immediately into cold three-per cent metaphosphoric acid solution, ground in a blender under carbon dioxide, and made up to 250 c.c. in a volumetric flask. The cooking water was cooled by setting the beaker in cold water, three grams of dissolved metaphosphoric acid was added, and the solution was made up to 100 c.c. in a volumetric flask.

In cooking with steam pressure a three-quart aluminum pressure saucepan was used. The cooking temperature was regulated by means of a thermometer inserted through a rubber stopper in the opening left by removing the safety stopper. By preliminary tests it was found that cooking for five and one-half minutes at 117°C.(242.6°F.) was necessary to soften 25-gram pieces without causing the pieces to fall apart. Each sample was cooked separately by laying it on a watch glass placed on a wire rack in the pressure saucepan. At the end of the cooking period the pressure saucepan was set in a pan of cold water to reduce pressure rapidly, the cooker opened quickly, and the cooked sample transferred immediately to cold metaphosphoric acid solution. All cooked samples were ground in the blender under carbon dioxide and made up to suitable volume with three-per cent metaphosphoric acid solution.

Pressure cooking proved to be the best method of cooking from the standpoint of retention of ascorbic acid in the cooked potato, 83 per cent of the original ascorbic acid being present. Boiling for 20 minutes in 25-gram pieces proved the least advantageous of all the methods used, as only 69 per cent of the original ascorbic acid was found in the potato while 19 per cent was found in the cooking water. The addition of sugar as in candying seemed to have a slight advantage as 74 per cent of the original ascorbic acid was retained in the samples cooked by this method.

Baking and Boiling Whole: For baking and boiling, whole, uniform-sized potatoes were used without paring. Potatoes were baked for 45 minutes at 190.6°C.(375°F.). This time and temperature softened the potatoes throughout without excessive browning of the surface. The samples boiled whole were put into a large beaker containing enough boiling distilled water to keep them covered and boiled gently for 40 minutes. The baked or boiled potatoes were cooled on a wire rack at room temperature until cold enough to handle easily, and sampled by the method used for sampling the raw potatoes for variety and storage studies. The figures on ascorbic acid losses are based on the average ascorbic acid value of 10 cooked potatoes as compared with the value of 10 raw potatoes determined the day before. The baked potatoes were weighed before and after baking. The ascorbic acid value of the baked sweet potato was 0.216 milligram per

TABLE 3

Losses of Ascorbic Acid Owing to Cooking Porto Rico Sweet Potatoes

Method of cooking	Cooking time	Ascorbic acid		
		Retained in potato	Found in cooking water	Destroyed
	min.	*pct.*	*pct.*	*pct.*
25-gram pieces in 100 c.c. water	20	69.2	19.2	11.6
25-gram pieces in 100 c.c. water + 25 gm.	22	73.7	15.2	11.1
Pressure saucepan at 117°C.(242.6°F.), 25-gram pieces	5½	83.4	.....	16.6
Baked	45	75.7[1]	.....	.....
Boiled whole in skins	35	75.5	.....	.....

[1] Calculated on cooked weight of entire lot as compared with raw weight.

gram or 87 per cent of the original value of 0.248 milligram per gram. As the loss in weight during baking was considerable, results were recalculated and expressed (Table 3) on the basis of the raw weight of each lot of potatoes. When moisture loss on the whole sample was considered, only 76 per cent of the original ascorbic acid was found to be present. Potatoes boiled whole for 40 minutes in water to cover, showed an ascorbic acid value of 75 per cent of that of the raw potatoes which were analyzed at the same time (Table 3).

DISCUSSION

The results of this study are in harmony with those of other workers with the exception of Scoular and Eakle (1943), who report considerably lower values. The storage temperature for their samples was lower than for those reported in this study, 10°C.(50°F.) as compared with 15.6 to 26.7°C. (60 to 80°F.). In a personal communication the senior author (1943) of the above paper states, ". . . a commercial storage plant was used, and we had no means of knowing that the temperature quoted us was the actual temperature at which our sweet potatoes were stored." She further states, ". . . our samples all showed sprouting and other deterioration. In some cases, over 50 per cent of the stored samples were decomposed." It would seem that the storage temperature on their samples may at times have been low enough to cause damage. It has been shown by Kimbrough

and Bell (1942) that a structural breakdown accompanied by changes in flavor and color occur when sweet potatoes are subjected to a temperature of 4.4°C.(40°F.) or below. It is altogether likely that decrease in ascorbic acid content may occur as well. Other possible factors which may have contributed to such a wide discrepancy in results include differences in soil and climatic conditions under which the products were grown, varieties of potatoes tested, and difference in the method of sampling. There is undoubtedly need for further study on the effect of soil and climatic conditions and also the effect of storage temperature on the ascorbic acid value of common foods. Until such data is available the reasons for such discrepancies can only be surmised.

The results of cooking in the study of Scoular and Eakle also differ considerably from those obtained by the author. The use of the mechanical blender in the author's laboratory may have resulted in more complete extraction of the vitamin from the raw samples than was obtained by Scoular and Eakle whose samples were ground by means of the mortar and pestle. In addition different acid solutions were used for extraction by the two laboratories as Scoular and Eakle (1943) state that they followed the method of Mack and Tressler as modified by Bessey and King (1933). Reference to this method shows the use of hot eight-per cent acetic acid for extraction of vegetable samples. Either one of these two differences in procedure or a combination of both could account for low ascorbic acid values on the raw potatoes and consequent apparent increases of that value on cooking. Other factors which may have contributed to differences in the results of the two laboratories are a difference in the methods of sampling and the very different ascorbic acid content of the sweet potatoes used in the two studies.

SUMMARY

Sweet potatoes are a good source of ascorbic acid, and may be an important source of this vitamin in the diet of the South because of the quantities produced and consumed. Values during the first month after harvest ranged from 0.235 milligram per gram for the Triumph variety to 0.333 milligram per gram for the Nancy Hall variety.

The ascorbic acid content of sweet potatoes is a variety characteristic worthy of consideration in breeding.

Losses ranging from 28 to 40 per cent occur after long storage at 15.6 to 26.7°C.(60 to 80°F.). Varietal differences tend to become less significant after storage. After seven months' storage, values ranged from 0.166 milligram per gram for the Triumph variety to 0.203 milligram per gram for the Nancy Hall variety.

From 69 to 83 per cent of the original ascorbic acid value of sweet potatoes was found in the potato after cooking by accepted methods.

Losses owing to solution in cooking water were more significant than losses owing to heat destruction when boiling sweet potatoes in water.

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VARIETAL DIFFERENCES IN GEL STRENGTH OF STARCH IN NORTH DAKOTA HARD RED SPRING WHEATS[1]

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Methods of determining the strength of gelatinized starches have been described by Woodruff and MacMasters (1938) and Knowles and Harris (1943). Starch gels prepared from different strains of Illinois-grown corn were found to differ significantly by Woodruff and MacMasters. Knowles and Harris showed variability in starch gel strength when wheats from different classes were compared, although definite differences among hard red spring wheat varieties were not found. These workers applied the "tenderness tester," developed by Binnington, Johannson, and Geddes (1939), to the determination of wheat-starch gel strength. This is apparently a somewhat more sensitive apparatus than the Tarr and Baker jelly tester used by Woodruff and MacMasters (1938), as they found it impossible to evaluate wheat-starch gel strength with this equipment.

The investigation described in the present paper is a continuation of the work previously carried out at the North Dakota Experiment Station. The equipment described by Harris and Knowles (1940) and Knowles and Harris (1943) was used and the methods were essentially the same, with the exception that a fermentation cabinet with temperature and humidity control was employed to maintain constant temperature and humidity conditions while the starch was cooling and hardening.

EXPERIMENTAL PROCEDURE

Thirty-two samples of starch were prepared from experimentally milled, straight-grade, hard red spring wheat flours of approximately 70 per cent extraction and used in this study. The wheats were grown in 1942 at four stations situated at the following locations in the state: Fargo in the southeastern portion, Langdon in the northeastern, and Dickinson and Williston in the western half, Williston being the more northerly of the two by a considerable distance. The wheats were sound and free from damage of any kind. The varieties are representative of those being grown in the state, with the exception of two which are now under test for later commercial release. These varieties have not been named and are designated by number. Significant differences in mixogram properties among these varieties have been found by Harris and associates[2]; but it is improbable that mixing behavior is related to starch characteristics, inasmuch as the gluten has been found to carry the varietal mixing pattern, Swanson and Andrews (1943); Harris *et al.*[2]

[1] Published with the approval of the director of the North Dakota Agricultural Experiment Station.

[2] Data being prepared for publication.

The starches were prepared by methods already described by Harris and Knowles (1940) and Harris and Sibbitt (1941); no reagent other than 0.1 per cent sodium phosphate at a pH of 6.8 was employed in the preparation. The starches were not washed, but after centrifugation in an International Type SB centrifuge were dried on plates at 32.2° C. (90° F.) for approximately 15 hours, with fanning. Drying was facilitated by turning the starches two or three times with a spatula and breaking up any lumps formed during drying. The dried starch, at a moisture content of approximately 11 per cent, was passed once through a motor-driven Arcade coffee mill and analyzed for moisture and nitrogen content. The moisture was determined in an electric drying oven for one hour at 130°C.(266°F.) ± two degrees C. Nitrogen was found by the Kjeldahl-Gunning procedure.

The gelatinization was accomplished by heating a six-per cent (dry basis) starch suspension to 95°C.(203°F.) in a constant-temperature bath held at 120°C.(248°F.). Distilled water at laboratory temperature was used. This was added in a stream from a pipette to the starch contained in a 200-ml. Erlenmeyer flask with a swirling motion to prevent lumps from forming. The flask and contents were also shaken with a rotary motion during gelatinization. About seven minutes were required to bring the starch suspension to the desired temperature.

Following gelatinization the starch was poured into petri dishes of uniform size, 75 mm. in diameter and 10 mm. in depth, and allowed to stand at least 20 hours at 30°C.(86°F.) and 73 per cent relative humidity in the cabinet. The following morning the tenderness curves were obtained by the method described by Knowles and Harris (1943). Nine replications were made on each individual starch, and if results appeared to be unusual further tests were made.

Baking determinations were run on the flours using the malt-phosphate-bromate [3] method with three-hour fermentation. One hundred grams of flour was used in each mixing. The different starches were also blended with a common gluten menstruum and baked according to the method outlined by Harris and Sibbitt (1941) at a uniform protein level of 13.5 per cent (13.5 per cent moisture basis). The baking formula was the malt-phosphate-bromate as before with seven per cent sugar. A micro-baking method using 25 grams of flour was employed in these blend bakings. The milling was done on a three-stand Allis-Chalmers mill at a room temperature of 21.1°C.(70°F.) and relative humidity of approximately 75 per cent, using the flow sheet described by Sibbitt, Scott, and Harris (1943).

DISCUSSION

Comparative quality data secured from the wheats with varietal designations and starch gel strengths are shown (Table 1). Wheat-protein content varies from 12.4 to 15.7 per cent, a range of 3.3 per cent, while flour protein ranges from 11.7 to 15 per cent, a difference of 3.3 per cent.

[3] This formula contains in addition to flour, water, yeast, salt, and sugar, 0.3 per cent high diastatic malt, 0.1 per cent ammonium phosphate, and 0.001 per cent potassium bromate.

TABLE 1

Comparative Data Relating to the Investigation

Sample No.	Variety	Protein content [1]		Loaf volume	Gel [2] strength	Loaf [3] volume
		Wheat	Flour			
		FARGO				
		pct.	*pct.*	*c.c.*	*sec.*	*c.c.*
42-275	Thatcher	14.2	13.6	715	47	125
276	Rival	14.6	13.5	625	48	115
278	Pilot B	14.7	13.4	655	59	135
280	Vesta	14.2	13.5	615	70	130
283	Renown	15.5	14.1	690	39	140
284	Regent	15.3	14.3	730	50	140
293	2822	15.7	14.7	735	67	135
294	2829	14.8	13.5	710	38	135
		DICKINSON				
42-300	Thatcher	12.7	11.8	575	41	150
301	Rival	13.4	12.4	585	40	155
303	Pilot B	13.1	11.7	600	50	160
305	Vesta	12.4	12.0	565	50	140
308	Renown	13.5	12.8	630	43	150
309	Regent	13.6	12.9	655	43	135
321	2822	13.9	12.9	640	47	115
322	2829	13.2	12.3	610	37	115
		LANGDON				
42-325	Thatcher	13.7	12.8	680	33	125
326	Rival	14.5	13.1	700	35	110
328	Pilot B	14.2	12.9	680	45	125
330	Vesta	14.4	13.1	655	46	115
332	Renown	15.0	13.8	735	29	125
333	Regent	15.1	14.2	770	43	130
339	2822	14.7	13.7	705	43	110
340	2829	14.6	13.3	645	39	120
		WILLISTON				
42-344	Thatcher	14.9	13.8	600	43	110
345	Rival	13.9	12.9	605	35	125
347	Pilot B	14.7	13.5	655	45	120
348	Vesta	15.3	13.9	600	53	125
350	Renown	15.6	14.5	680	43	130
351	Regent	15.7	15.0	695	38	130
354	2822	15.2	13.8	675	50	115
355	2829	14.5	12.9	635	35	110

[1] Expressed on 13.5 per cent moisture basis. [2] Gel strength is measured by the number of seconds required to break the surface of the starch gel. [3] Obtained from baking 25-gram blends of different starches with a constant gluten substrate at a uniform protein level of 13.5 per cent.

Loaf volume of the flours varied from 565 to 770 c.c. or a difference of 205 c.c. The loaf volume of the starch-gluten blend bakings, however, range from 110 to 160 c.c., a difference of 50 c.c. No relationship is evident between these loaf volumes and gel strengths. The gel-strength values shown are the means of nine replicates which varied considerably among themselves. The means range from 29 to 70 seconds.

Gel-strength averages were calculated for the various wheats and starches included in the study (Table 2), while the same data are shown graphically (Fig. 1). The significance of differences in these average values will be discussed in the following paragraphs.

TABLE 2

Average Gel-Strength Readings for Varieties and Stations[1]

Varieties	Gel strength
	sec.
Vesta	55
2822	52
Pilot B	50
Regent	43
Thatcher	41
Rival	40
Renown	38
2829	37
Stations	
Fargo	52
Dickinson	44
Williston	43
Langdon	39

[1] Arranged in order of decreasing gel strength.

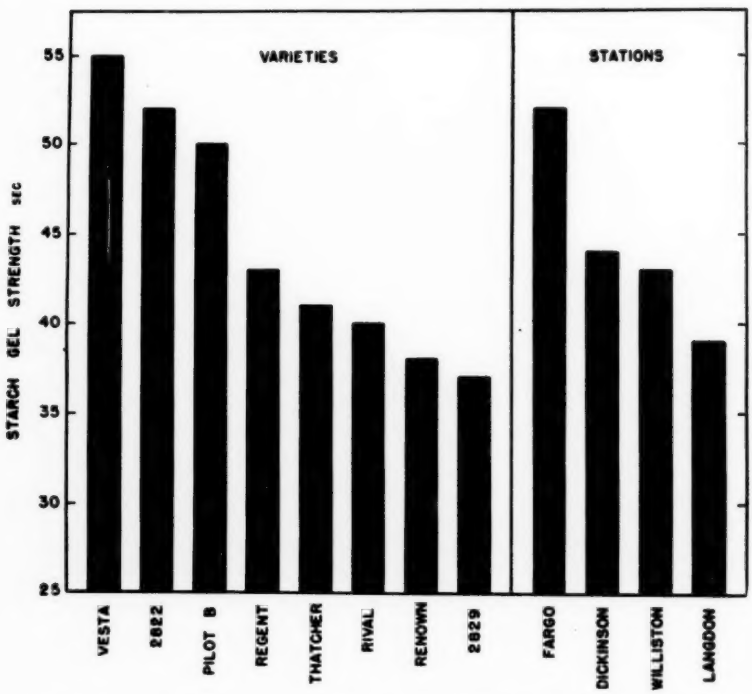

FIG. 1. Comparative wheat-starch gel strengths among varieties and stations.

An analysis of variance of the starch-gel-strength data was made (Table 3). The interactions, with the exception of varieties × stations, were grouped together and used as the error term, inasmuch as the interaction with replicates would appear to have little meaning. The replicate variance was calculated by methods developed by R. A. Fisher. Since the purpose of the experiment was to ascertain differences in gel strength among wheat varieties over the whole area that the stations sample, the stations can be thought of merely as replications. The results may now be quickly summarized.

TABLE 3

Analysis of Variance of Starch Gel Strength[1]

Source of variation	Sums of squares	Degrees of freedom	Variance	F	5-per cent point	1-per cent point
Between varieties	11,276	7	1,610.86	25.86	2.05	2.73
Between stations	6,867	3	2,289.00	36.74	2.65	3.88
Between replicates	3,282	32	102.50	1.64	1.51	1.77
Interaction (varieties × stations)	4,551	21	216.71	3.48	1.61	1.94
Error	13,958	224	62.30	.....	.....	.....
Total	39,934	287	.....	.....	.....	.....

[1] Gel strength measured in seconds.

There is a very significant difference among variety means. A difference of 3.7 seconds between gel-strength means is significant at the five-per cent point. Glancing at the data (Table 2) it will be seen that many of the means differ significantly. Similarly there is a significant difference between station means, a difference of 2.6 seconds being significant. Fargo is higher than the other stations, while Langdon is lowest (Table 2 and Fig. 1). These results for the stations agree with the findings of Knowles and Harris (1943). There is no significant difference between the mean gel strengths of the wheats grown at Dickinson and Williston. The difference between replicates is just significant, while the interaction of varieties and stations is very significant. This shows that the gel strengths of the wheat varieties vary among themselves at one locality in a significantly different manner than at another locality.

The data secured in this study show that the gel strengths of starches prepared from different varieties of hard red spring wheat differ significantly. It is also apparent that environmental factors of soil and climate affect those starch properties that are responsible for gel strength, as this property varied significantly among stations, while the individual varieties did not react in the same manner under different environments. The relation of these variations in gel strength with baking quality is not evident in the data. Knowles and Harris (1943) have pointed out that no relationship apparently exists between these two properties.

SUMMARY AND CONCLUSIONS

Thirty-two samples of starch were prepared from flours milled experimentally from eight varieties of hard red spring wheat. These wheats were grown under comparable conditions at four North Dakota stations

in 1942. The starches were dried and analyzed for moisture and nitrogen content. Six per cent (dry-matter basis) suspensions of the starches in distilled water were gelatinized at 95°C.(203°F.), then poured carefully into petri dishes, and allowed to stand for approximately 20 hours at 30°C. (86°F.) and 73 per cent relative humidity. The gel strengths were then determined by means of a tenderness tester, using the method described by Knowles and Harris (1943).

The data secured, in terms of seconds required to rupture the gel, were subjected to statistical analysis. Extremely significant differences were found among varieties and also among stations. It is evident that some varieties of hard red spring wheat produce starches of higher gel strength than other varieties. It is also apparent that environment during the formation of the kernel has an influence upon this property of wheat starch.

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RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENTS OF GRAIN SORGHUMS

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The grain sorghums, including milo, kafirs, hegari, feterita, and others, are of great importance as a source of feed in the semidry areas found in many parts of the Southwest. Over 8,000,000 acres, producing above 86,000,000 bushels of seed, valued at more than $47,000,000, have been reported in recent years. Some of the varieties have a sweet juice and are used for forage and silage and, in some cases, for syrup making; but, for the most part, they are raised as grain for poultry and livestock. Heller and Green (1926) reported the average chemical content of many of the Oklahoma-grown sorghums. The vitamin A, as recognized at that time, was determined for many varieties. A few years later Smith (1930) reported on the nutritive value of some varieties grown in Arizona. Since that time, however, many new varieties have been introduced, and our knowledge of the vitamin B complex has been materially changed. In view of the fact that the sorghums will undoubtedly be more extensively used both for industrial and human consumption, it seemed advisable to determine the relative distribution of some of the important members of the B complex.

EXPERIMENTAL PROCEDURE

In 1941 and 1942, 29 varieties of sorghums were selected and planted both at Perkins, Oklahoma, in sandy loam, near the center of the state and at Woodward in the drier western section. The sorghums were produced in varietal plots under comparable conditions. In the fall when the seed was completely developed, 20 representative heads were selected from each variety, transferred to the laboratory, dried, hand threshed, and carefully separated from hulls. The seeds were then ground and stored in a cool, dark room in sealed containers until analysis was completed.

VITAMIN DETERMINATIONS

Both chemical and microbiological methods were repeatedly tried, and the microbiological method seemed to be the most suitable. The following procedure was used.

The riboflavin content was determined by the microbiological method of Snell and Strong (1939) in which the response of *Lactobacillus casei* ϵ to riboflavin is measured by titrating the acid produced. The samples were prepared for analysis as follows: One or two grams of finely ground grain were extracted with 50 ml. 0.1 N HCl and 25 ml. H_2O at 15 pounds

TABLE 1

Vitamin Content[1] of Oklahoma-Grown Grain Sorghums

Sorghums	Riboflavin				Niacin				Pantothenic acid			
	Perkins		Woodward		Perkins		Woodward		Perkins		Woodward	
	1941	1942	1941	1942	1941	1942	1941	1942	1941	1942	1941	1942
Standard kafir	1.32	1.60	1.44	1.33	33.6	33.35	31.7	28.75	14.07	15.0	12.19	10.32
Sharon kafir	1.26	1.42	1.40	1.52	36.8	36.0	32.0	25.0	15.63	13.13	15.32	13.44
Sunrise kafir	1.24	1.98	1.41	1.31	38.5	37.3	37.1	37.2	13.12	13.13	12.00	10.47
Reed kafir	.....	.....	1.42	1.66	.....	.....	33.8	28.75	.....	.....	15.00	12.75
Hydro kafir	.....	1.50	.....	.....	.....	35.5	.....	.....	.....	12.91	.....	.....
Rice kafir	.....	1.38	.....	.....	.....	29.0	.....	.....	.....	12.75	.....	.....
Red kafir[2]	1.52	1.17	.....	.....	25.4	39.1	.....	.....	13.39	11.88	.....	.....
Sedan red kafir	.....	.....	1.44	.....	.....	.....	35.0	.....	.....	.....	14.69	.....
Darso[3]	1.20	1.42	1.35	1.42	22.0	20.5	22.3	21.5	13.43	13.09	11.57	12.75
White darso, Wd. No. 52-29	1.25	1.61	1.42	1.30	31.7	35.5	24.7	29.9	15.31	13.91	12.00	11.25
Yellow darso, Wd. No. 36-12	1.31	1.58	1.54	1.19	35.1	35.9	27.5	29.1	14.84	14.06	14.07	12.34
Feterita, C.I. No. 182	.....	.....	1.44	1.96	.....	.....	51.9	43.75	....	.....	15.00	15.38
Cache feterita	1.49	1.47	.....	.....	52.9	56.25	.....	.....	13.59	12.44	.....	.....
Kaferita, C.I. No. 812	1.31	1.45	.....	.....	49.35	50.9	.....	.....	11.26	13.75	.....	.....
Chiltex	.....	.....	1.36	1.92	.....	.....	55.2	43.0	.....	.....	12.75	13.39
Hegari, C.I. No. 750	1.70	1.17	1.51	1.64	61.0	50.0	55.6	53.0	13.75	12.44	13.93	11.85
Early hegari, S.A. 281	.....	.....	1.62	2.04	.....	.....	50.7	46.0	.....	.....	15.88	15.80
Migari, Wd. 11-2	2.07	.....	1.60	1.46	83.6	.....	78.75	73.65	12.17	.....	11.79	11.26
Dwarf yellow milo, C.I. No. 332	.....	.....	1.32	2.06	.....	.....	61.6	50.5	.....	.....	12.87	13.84
Dwarf yellow milo, T.S. No. 338	.....	.....	.....	1.98	.....	.....	.....	60.6	.....	.....	.....	15.88
Finney milo	.....	.....	1.48	.....	.....	.....	53.5	.....	.....	.....	13.95	.....
Schrock	.....	1.28	.....	.....	.....	31.75	.....	.....	.....	14.38	.....	.....
Leoti sorgo	.....	2.08	2.04	1.42	.....	38.1	37.5	29.0	.....	14.32	12.88	11.84
Sumac sorgo, F.C.I. No. 1712	.....	1.73	1.68	1.42	.....	32.5	35.8	28.0	.....	13.44	13.44	13.44
Sugar drip sorgo	.....	1.29	1.61	1.62	.....	24.7	22.4	24.5	.....	13.44	13.85	14.38
Atlas sorgo	.....	1.99	1.84	1.94	.....	32.5	37.6	34.0	.....	14.12	13.74	12.75
Sourless sorgo (African millet)	.....	1.52	.....	1.48	.....	22.0	.....	22.5	.....	14.34	.....	15.25

[1] Given in micrograms per gram. [2] In 1941, Ks. No. 37 M 133; in 1942, 866 x 71-5. [3] At Perkins, T.S. No. 28; at Woodward, C.I. No. 615.

pressure for 15 minutes. This extract was neutralized with NaOH using brom thymol blue as an external indicator and diluted to 200 ml. The solid material was allowed to settle or was centrifuged out. Three to five ml. of this extract were used in the assay tubes. Duplicate determinations were made at two or more levels of concentration. The recovery of added riboflavin ranged from 90 to 120 per cent.

The determination of niacin was made by the method of Snell and Wright (1941). By this method the response of *Lactobacillus arabinosus* 17-5 to niacin is measured by titrating the acid produced. The samples were prepared for analysis by treating with NaOH and autoclaving by the method of Tepley, Strong, and Elvehjem (1942). One-tenth to five-tenths of a gram of finely ground sample was used and the extract was diluted to 200 ml. Two to five ml. of this extract were used. Duplicate determinations were made at these levels. Recovery of added niacin ranged from 86 to 112 per cent.

Pantothenic acid was determined by the microbiological method of Pennington, Snell, and Williams (1940), in which the response of *Lactobacillus casei* ϵ to pantothenic acid is measured by titrating the acid produced. The sample was prepared for analysis by the method of Tepley, Strong, and Elvehjem (1942). Recovery of added pantothenic acid ranged from 90 to 120 per cent.

An inspection of the averaged results (Table 1) shows that there is little difference in the riboflavin content of various sorghums and the average is comparable to other cereals of similar structure. The niacin varies as much as 100 per cent within the group. It is interesting that the varieties with crypto-brown and milo-yellow pericarps, also club kafirs, have a niacin content much higher than the other varieties. The average is somewhat less than wheat but higher than many cereals. The pantothenic acid variation between samples is small and the average content is comparable to that of other grains.

SUMMARY

The riboflavin, niacin, and pantothenic acid contents of 29 varieties of sorghum raised in two locations in Oklahoma in 1941 and 1942 are presented. The variation in riboflavin and pantothenic acid is small within the groups and compares favorably with other cereals. The niacin content of the brown-coated varieties is much higher than that of the other members.

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VITAMIN B-COMPLEX STUDIES ON DEHYDRATED MEATS[1]

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Dehydration as a method of preserving foods has been emphasized recently by the need for stable and concentrated foodstuffs. Materials heretofore processed in other ways are now being dried in great quantities in an effort to fill domestic and export demands. In common with most other processing methods, dehydration modifies the nutritive values of foods. It is imperative that we know the nature and extent of these changes. This is especially true of meat, which ordinarily contributes important amounts of protein and of the B-complex vitamins to the American diet.

For dietary calculations only the nutritive value of the final product need be known, but from a more comprehensive scientific standpoint it is important to study the changes occurring during processing. Poling, Schultz, and Robinson (1943) have shown that the dehydration process does not cause any significant protein deterioration. The present investigations were carried out to determine the extent to which the vitamins of beef and pork are retained during dehydration in plant-scale operations. In general, vitamin losses have been found to be similar to those taking place during cooking [McIntire, Schweigert, Henderson, and Elvehjem (1943)]. During the drying operation itself surprisingly little loss occurs.

Although the methods of meat dehydration are generally known, and have been recently reviewed by Kraybill (1943), a brief outline of the procedure which was followed with the samples under consideration will aid in interpreting the data. With both beef and pork the initial step is the precooking of one- to two-thousand-pound lots of boned and cubed meat in steam-jacketed kettles. The meat is stirred occasionally during this operation. Pork is cooked for 30 minutes after reaching an internal temperature of 73.9°C.(165°F.); beef is usually cooked a few minutes longer so as to remove more of the moisture. Internal temperatures of the products at the end of the precook have risen to 82.2°C.(180°F.). Following the precook, beef is ground and then dried in a current of warm air in a rotary drier. Pork is handled differently. During cooking it yields a broth which is drawn off, skimmed to remove fat, and then concentrated under a 24-inch vacuum to a solids content of approximately 50 per cent. This concentrated broth is later reincorporated with the pork after the latter has been dried in the same type of drier as the beef. By adding calculated quantities (based on moisture and fat analysis) of the fat which had been previously separated, the remixing step may also

[1] The data in this paper were presented before the Division of Agricultural and Food Chemistry of the American Chemical Society at the Detroit meeting, April 12, 1943.

be used to adjust the fat and moisture content of the dehydrated pork to the desired level.

EXPERIMENTAL PROCEDURE AND DISCUSSION

In studying the vitamin losses during these processes, samples of the product were taken during each stage and assayed for moisture, protein, and fat as well as for the vitamins under consideration. Obviously, proper sampling is of prime importance since different parts of many animals are included in each batch of raw meat. In an effort to secure samples representative of such a heterogeneous charge, small slices were cut from pieces of the meat as it was being fed into the cooker. Part of these slices were taken with the grain of the meat and part across the grain. Some were taken through the center of each type of cut and others were taken from an end or edge. The relatively large amounts of meat so obtained were then ground, mixed thoroughly, reground, and remixed until a homogeneous mass resulted. A portion of this was reserved for assay. Sampling of the cooked meat was much simpler since it could be effected during grinding by continuously collecting small portions of the product at the grinder head. After thorough mixing a small amount typical of the entire lot was taken as a sample. Aliquots of the dehydrated meat, already well mixed during drying, were always taken from product which had been thoroughly remixed. The broth, drained from cooked pork, was sampled by collecting a portion of the aqueous solution. The fat was disregarded after preliminary assays had indicated that it was almost devoid of the water-soluble vitamins.

The thiamin values herein reported were determined by the thiochrome procedure as outlined by Conner and Straub (1941). Riboflavin, niacin, and pantothenic acid were assayed microbiologically upon samples which had been enzymatically hydrolyzed as recommended by Cheldelin, Eppright, Snell, and Guirard (1942). In preparing the ground, raw samples for assay, 100-gram portions were homogenized with 200-gram quantities of water in a Waring blender. The need for this step is obvious when one considers the fact that an aliquot of two grams must represent 1,000 to 2,000 pounds of meat. The ground and dried products were blended with greater portions of water so that the final emulsions contained approximately the same proportion of solids as those prepared from raw meats. In order to prevent any variation in standards from affecting the values for percentage recovery of the vitamins, all samples of any one series of determinations were run simultaneously.

With each type of meat the vitamin content per gram of protein and the per cent retention at the various stages of dehydration were quite constant for a number of runs. Hence, data for typical series of samples may be considered as representative of the changes to be expected; such data for beef are presented (Tables 1 and 2). The percentage of moisture, protein, and fat in the beef at the various stages of processing are indicated (Table 1) along with the values for thiamin content per gram of sample and per gram of protein, and for the per cent retention (based on the content per gram of protein) after cooking and drying. Similar computations are shown for riboflavin, niacin, and pantothenic acid

(Table 2). Considering the relatively mild precook given the meat, all of these values seem quite reasonable, except, of course, the 105-per cent retention of riboflavin.

These 100-plus values for riboflavin have been consistently obtained, a fact which we attribute to the formation of stimulating substances during cooking. This seems the most logical explanation since the aqueous extract from raw samples gives identical values with or without petroleum ether washes while the aqueous extract from cooked samples yields lower values after ether washes. This would indicate the development of some stimulatory substance for *Lactobacillus casei*[2] during the heat treatment, presumably of a fat-soluble nature, Strong and Carpenter (1942) and Bauernfeind, Sotier, and Boruff (1942). Treatment with papain and taka-diastase does not increase the riboflavin value for raw samples but does reduce the value for cooked samples. This is being further investigated.

TABLE 1

Thiamin Content of Beef During Dehydration

Sample 176	Moisture	Protein	Fat	Thiamin		
				In sample	In protein	Retention
	pct.	*pct.*	*pct.*	*μgm./gm.*	*μgm./gm.*	*pct.*
Raw beef	71.1	19.81	8.7	0.62	3.13	....
Cooked beef	46.5	32.31	18.9	0.92	2.85	91
Dehydrated beef	6.1	58.63	31.5	1.39	2.37	76

Calculations of the per cent of vitamin retention at the various stages during dehydration of pork are much more complicated than they are for beef because of the separation of the material into three fractions after cooking. The division of the product makes calculation to a solids or protein basis illogical; hence, retentions have been computed on a "total" basis. In applying this method the total amount of a vitamin at each step in the process is found by multiplying the weight of the product by the concentration of the vitamin per unit of weight, after which the per cent retention may be found by a simple proportion with the amount in the raw meat. When it can be applied, this procedure is perhaps more satisfactory than any other since changes in the fat, protein, and moisture relationships will not interfere with interpretation of the results. Unfortunately, this method of "totals" is applicable only when both the vitamin concentration and the total weight of each product can be measured. Since the actual drying operation for meat is continuous, it is impossible to determine directly the weight of dehydrated product corresponding to a given amount of cooked meat. The same difficulty arises with the broth, which must be pooled with that from other lots and concentrated in large batches for effective operation of the vacuum still. However, the quantity of dehydrated meat yielded by a given amount of cooked meat can be

[2] The quantities of riboflavin present in the various products were determined by measuring the amount of acid produced by this organism in the presence of aliquots of the samples.

TABLE 2

Riboflavin, Niacin, and Pantothenic Acid Contents of Beef During Dehydration

Sample 176	Riboflavin			Niacin			Pantothenic acid		
	In sample	In protein	Retention	In sample	In protein	Retention	In sample	In protein	Retention
	μgm./gm.	*μgm./gm.*	*pct.*	*μgm./gm.*	*μgm./gm.*	*pct.*	*μgm./gm.*	*μgm./gm.*	*pct.*
Raw beef	1.97	9.96	.....	49.5	249.5	....	3.40	171.5	....
Cooked beef	3.50	10.82	109	77.4	239.4	96	4.00	123.7	72
Dehydrated beef	6.16	10.51	105	134.0	228.5	92	6.81	116.1	68

TABLE 3

Thiamin Content of Pork During Dehydration

Sample 169	Pounds	Moisture	Protein	Fat	Thiamin			
					In sample	Total	In protein	Retention
		pct.	*pct.*	*pct.*	*μgm./gm.*	*gm.*	*μgm./gm.*	*pct.*
Raw pork	8,097	54.0	15.31	30.41	6.63	24.35	43.25	.....
Cooked pork	4,660	47.0	24.81	27.90	6.71	14.28	27.01	59.0
Broth	477	89.9	9.13	0	13.99	3.03	153.15	12.0
Fat	1,164	.....	0.19	.....	0.25	0.13	131.60	0.5
Cooked pork plus broth	5,137	.....	.....	.....	7.44	17.31	.....	71.0
Dried meat	(2,381)	10.3	48.81	40.60	11.90	12.82	24.41	52.0
Concentrate and broth	(96.6)	49.9	45.12	.....	63.42	2.78	140.01	11.0
Finished product (dried meat and concentrate)	(2,478)	.....	48.68	.....	13.83	15.60	28.42	63.0

accurately calculated by determining the total amount of protein in the cooked meat and dividing this by the percentage of protein in the dry product. Using this as the yield, the total amount of each of the vitamins remaining at this stage may be determined as indicated above. Similar calculations for the broth show the amount of concentrate yielded and its vitamin retention. The weights found by calculations from the protein data rather than by actual observation have been enclosed in parentheses (Table 3).

Theoretically the concentrate from each charge of meat is added back to the same batch, but in a continuous process this is not feasible. Furthermore, some lots of dehydrated meat are undesirably high in moisture and must be mixed with others low in moisture before the broth concentrate can be added to prepare a final product. Because of this mixing of portions which originated from different cookings, the data on any sample of the

TABLE 4

Thiamin Retention During Pork Dehydration

Lot. No.	Protein	Thiamin		
		In sample	In protein	Retention [1]
	pct.	*μgm./gm.*	*μgm./gm.*	*pct.*
169	48.68	13.83	28.42	66
264	44.60	11.50	25.79	61
236	46.72	12.20	26.16	62
051	48.05	12.98	27.01	64
052	54.65	12.88	23.58	56
053	53.45	12.88	24.14	57
184	48.98	14.80	30.28	71
				Av. 63

[1] Using 42.45 micrograms per gram of protein as an average value for this type of pork as a basis for calculation.

final product cannot be referred directly to any one lot of raw meat. In order to obtain recovery data for the finished product, however, it is only necessary to total the percentages of retention in the dried meat and in the concentrate, since the mixing operation has no effect on the vitamin levels.

When these calculations have been carried out for thiamin, as indicated (Table 3), the over-all retention is found to be 63 per cent of the amount in the raw meat. This retention is approximately the same as that found by McIntire and co-workers (1943) for roast pork loin or hams. The greatest part of the loss occurs during the precook period. It is interesting to note that more than a fifth of the thiamin remaining after cooking is in the broth. When the processing operations have been completed, all of the protein originally present in the raw meat reappears in the finished product except for an insignificant amount lost with the fat. Hence, the over-all yield may be calculated on a protein basis. When this is done for Lot 169, for which detailed data are presented (Table 3), the retention is found to be 66 per cent as compared with 63 per cent obtained by the more elaborate method. That these values are typical of the product may be seen by reference to the protein and thiamin data for six other lots of

TABLE 5

Riboflavin, Niacin, and Pantothenic Acid Retention During Pork Dehydration

Sample 169	Riboflavin				Niacin				Pantothenic acid			
	In sample	Total	In protein	Retention	In sample	Total	In protein	Retention	In sample	Total	In protein	Retention
	μgm./gm.	*gm.*	*μgm./gm.*	*pct.*	*μgm./gm.*	*gm.*	*μgm./gm.*	*pct.*	*μgm./gm.*	*gm.*	*μgm./gm.*	*pct.*
Raw pork	2.21	8.13	14.43	.....	43.7	151.10	28.53	....	4.26	15.62	28.2	.....
Cooked meat	3.30	6.98	13.27	86	48.8	97.80	147.90	65	.....	.....	.....	.....
Aqueous broth	7.89	1.71	86.50	21	65.0	34.84	713.00	23	.....	.....	.....	.....
Fat	0.04	.....	.....	.....	.....	.....	.....	....	.....	.....	.....	.....
Total cooked (cooked meat plus aqueous broth)	.....	8.69	.....	107	.....	132.64	.....	88	.....	.....	.....	.....
Dried meat	6.20	6.72	12.71	83	98.4	104.35	201.60	69	.....	.....	.....	.....
Concentrate	.....	1.71	86.50	21	26.8	35.00	595.10	23	.....	.....	.....	.....
Finished product	7.48	8.43	15.36	104	124.1	139.35	254.70	92	10.15	11.41	20.8	73

dehydrated pork (Table 4). Each of the samples selected for assay was a composite of an entire day's run. While the value for thiamin per gram of protein in the raw pork going into these lots is unknown, we may assume a value of 42.45 micrograms per gram, this being the average of the values found for five similar lots of raw pork. The range for these samples was 41.17 to 44. At best, these recovery values are estimations, but they show the range of recoveries actually attained. Obviously, occasional samples with which little concentrate has been remixed will be lower in thiamin.

Calculations similar to those used in showing the retention of thiamin at the various stages indicate over-all recoveries of 92 and 104 per cent, respectively, for niacin and riboflavin. Only a few determinations of pantothenic acid were made on the raw pork and on the finished product of some samples. The values for this lot indicate a 74-per cent retention of the vitamin, which agrees well with the 68-per cent retention found during the dehydration of beef.

Comparison of retentions of the vitamins in pork with those for beef show remarkably good agreement. On the whole, the losses during dehydration are no greater than those which would occur during cooking and are, especially with respect to thiamin, much less severe than in canning. These determinations of vitamin retentions are, of course, only one phase of a study of the nutritive value of dehydrated meats. Of equal or greater importance is the determination of the stability of these vitamins during storage of the products as packed for shipment. Preliminary data with a limited number of samples indicate that there will be appreciable loss of thiamin during one to two months' storage at 26.7°C.(80°F.) and severe losses at higher temperatures. Whether these losses, as measured by the thiochrome method, reflect the true decrease in thiamin potency or whether they include some binding of thiamin in a nonextractable form, as suggested by Lane, Johnson, and Williams (1942), has not been decided. Much more work must be done on this phase of the problem before the final answer can be reported. Naturally this research is being pushed toward completion as rapidly as possible.

While less attention has been paid to the retention of the other vitamins during storage, the few assays that have been made indicate little or no loss of niacin, riboflavin, or pantothenic acid even during storage for several months at temperatures as high as 60°C.(140°F.).

SUMMARY

The thiamin, riboflavin, niacin, and pantothenic acid contents of pork and beef were determined at the various stages of dehydration.

Dehydrated beef retains 76 per cent of the thiamin, 105 per cent of the riboflavin, 92 per cent of the niacin, and 68 per cent of the pantothenic acid of the raw meat.

Dehydrated pork retains 63 per cent of the thiamin, 104 per cent of the riboflavin, 92 per cent of the niacin, and 73 per cent of the pantothenic acid of the raw meat.

Storage losses of thiamin in dehydrated pork are extensive at temperatures above 80°F.

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EFFECT OF REFRIGERATION ON RETENTION OF ASCORBIC ACID IN VEGETABLES[1]

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The problem of vitamin retention in vegetables from the time of harvest to the time of consumption is increasing in importance. Little work has been done on this problem except that concerned with preservation by freezing. It has been suggested by Fenton (1940) that losses of vitamin C might well be used as an indication of general decline in the nutritive value of the vegetables. It is on this theory that we base our present study of ascorbic acid retention in vegetables under various conditions of storage.

Wheeler, Tressler, and King (1939) studied vitamin C content and retention in various green leafy vegetables. They found that refrigeration reduced the loss of vitamin C in broccoli and spinach but not in lettuce. Spinach lost one-half of its original vitamin C content in three days' storage at room temperature but retained its ascorbic acid at temperatures slightly above freezing. Yaroshenko (1938) reported 30 to 50 per cent loss of vitamin C in spinach stored at room temperature for two days, while storage of the same vegetable at 2°C.(35.6°F.) reduced the loss to 10 to 17 per cent. Harris, Wismann, and Greelie (1940) studied the efficiency of different types of mechanical refrigerators and reported that the refrigerator with high humidity and low air movement gave favorable retention of ascorbic acid in the vegetables studied.

The present study was made on ascorbic acid retention in chard, lettuce, spinach, broccoli, and green beans. These vegetables were chosen because they are good sources of vitamin C and are sensitive to changes in the conditions of storage. Both home and store models of ice and mechanical refrigerators were used.

EXPERIMENTAL PROCEDURE

The first approach to this problem was made by determining the loss of vitamin C immediately after harvesting the vegetables. Samples of the different vegetables were placed in the acid-extraction solution at the garden while other samples were packed in crushed ice and taken to the laboratory within one hour. Assays were made on all samples immediately upon arrival in the laboratory. Those samples packed in crushed-ice containers were held for further storage in the containers both at room temperature and at a temperature of 6 to 7°C.(42.8 to 44.6°F.). Samples were removed from the crushed ice and placed in the hydrator of a

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We are indebted to Earl and Loraine Cardinal for carrying out some of the studies reported in this paper.

mechanical refrigerator, in a wire basket in the bottom of an ice refrigerator, and on a table in the laboratory.

Additional work was done during the fall and winter months when home-grown vegetables were not available; therefore many of the samples used were shipped to Madison from California. In all cases they were shipped in iced crates and the first samples were taken as soon as the vegetables arrived at the warehouse. These samples were then divided into smaller portions to be stored in the laboratory at room temperature and in the mechanical and ice refrigerators both on the shelf and in the hydrator. The mechanical refrigerator was kept at a temperature of 8 to 10°C. (46.4 to 50°F.), which is comparable to that used in most household refrigerators. The temperature of the ice refrigerator was slightly higher, 10 to 12°C. (50 to 53.6°F.).

Studies were also made on the retention of vitamin C in the vegetables during storage in a retail store. For this work both mechanical and ice refrigerators were used. The vegetables to be considered were stored for a period of 24 to 48 hours at a retail store and then removed and subjected to the various types of storage in the laboratory as described above.

A separate study was made of the ice display cases now used in many retail stores. Samples were stored directly on the crushed ice in the top of such a case, in the top of the case but not directly on the ice, and in the bottom of the case.

All assays were based on the original weight of the sample by determining the dry weight of the vegetable before and after storage. An approximate 40-gram sample was taken from each lot of the vegetables, and from this large sample three one-gram samples were selected. An attempt was made to make these three samples representative of the original large samples. The mean value for the three determinations was used in all cases and was reported as milligrams of ascorbic acid per 100 grams of sample. In most of the determinations there was less than a five-per cent deviation from the mean value. Typical results for a fresh sample of broccoli were 95, 93, and 94 mgm. of vitamin C per 100 gm. for the three samples.

All samples were assayed for vitamin C by grinding a weighed portion with acid washed sand using a 3¼-per cent metaphosphoric acid solution for extraction, Bessey (1938). Each sample was extracted three times with acid and this extraction mixture was then filtered through Whatman No. 40 filter paper. The filtrate was brought up to a known volume with 3¼-per cent metaphosphoric acid solution. The size of the aliquot used was dependent upon the concentration of the ascorbic acid in the solution and was diluted to a volume suitable for reading in the photoelectric colorimeter.

A standardized solution of 2,6 dichlorophenolindophenol buffered with sodium acetate was used for the determination in an Evelyn photoelectric colorimeter. The extracting solution was not buffered, for a low pH protects the vitamin C from oxidation during extraction while the buffered dye prevents the acid reduction of the dye during the period of reading in the colorimeter.

Colorimeter readings were made after 15, 30, 60, and 120 seconds. Most of the values fell within the range of two to five micrograms of ascorbic acid per cubic centimeter, suggested by Hochberg, Melnick, and Oser (1943) as giving accurate colorimetric readings. The method of Woessner, Elvehjem, and Schuette (1939) was used to extrapolate to zero time. Blank readings were made to correct for turbidity in each sample by decolorizing the dye-acid mixture in the colorimeter tubes with crystals of the pure vitamin C.

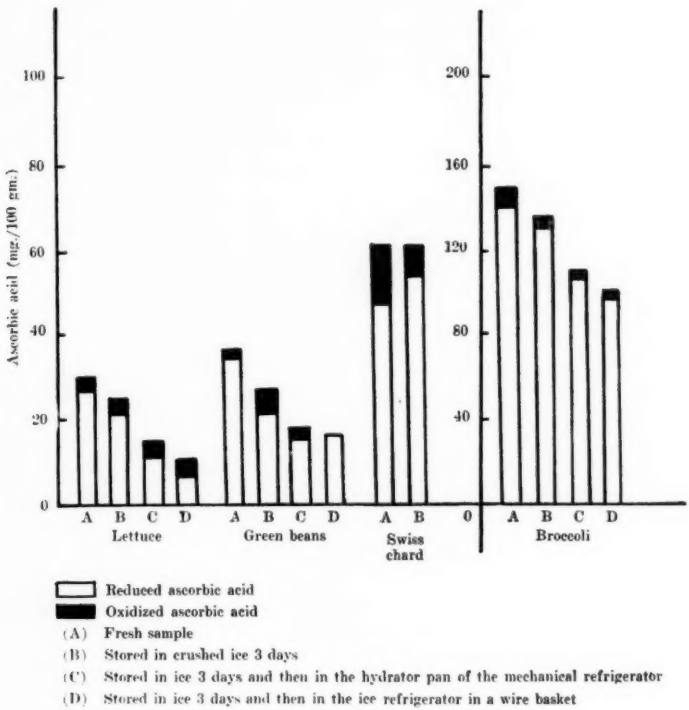

FIG. 1. Storage of vegetables in crushed ice.

Hochberg, Melnick, and Oser (1943) emphasize the importance of determining dehydroascorbic acid in the studies on vegetable storage. In our earlier work reduced and total ascorbic acid determinations were made; however, in the later studies we were interested primarily in the relative loss of vitamin C and determined only the reduced form so that we might be able to store and analyze more samples in a given period of time. Therefore, unless specified otherwise, the vitamin C values reported are of the reduced form.

When determinations of the total ascorbic acid were made, hydrogen sulfide was passed through a portion of the first metaphosphoric acid filtrate for 15 minutes. This solution was covered and allowed to stand

in the dark at room temperature for eight hours. Wet, oxygen-free nitrogen was passed through this solution for one hour. The total ascorbic acid was then determined colorimetrically as described above.

STORAGE IN CRUSHED ICE COMPARED WITH ROOM TEMPERATURE

Packing the vegetables directly in ice as they were harvested was found to be effective in preventing any loss of vitamin C during the first hour. The effect of storage in crushed ice on the retention of total and reduced ascorbic acid in the four vegetables studied (Fig. 1) shows that after three days' storage in the crushed ice broccoli, Swiss chard, and lettuce retained most of their vitamin C while green beans showed some loss.

Leaf lettuce packed in crushed ice, the container then kept in the cold room at 6 to 7°C., may be stored six days with less than a 10-per cent loss in the total ascorbic acid (Table 1). However, storing similar samples of

TABLE 1

Retention of Ascorbic Acid in Lettuce Packed in Crushed Ice Containers Stored at Room Temperature and in Cold Room[1]

Time (days)	Container kept at room temperature			Container kept at 6 to 7°C.		
	Reduced asco-bic acid	Oxidized ascorbic acid	Total ascorbic acid	Reduced ascorbic acid	Oxidized ascorbic acid	Total ascorbic acid
0	25	3	28	43	2	45
1	23	3	26	41	2	43
2	20	3	23	....	....	....
3	15	7	22	42	1	43
4	13	11	24	....	....	....
6	....	....	....	40	0.5	40

[1] Units expressed in mgm. per 100 gm.

lettuce in iced containers kept at room temperature resulted in a 20-per cent loss of vitamin after two days but the loss did not increase during the next two days. In the former sample, where the container was stored in the cold room, a very small amount of the ascorbic acid was found to be in the reversibly oxidized form and this amount decreased as the period of storage increased. In contrast to this the storage of similar iced containers of lettuce at room temperature resulted in a progressive increase in the dehydroascorbic acid formed as the storage period increased.

The former retail practice of sprinkling the room-stored lettuce to keep it "fresh" was not effective in preserving the total ascorbic acid (Table 2), but it did appear to decrease the amount which was found in the oxidized form. In the lettuce stored under ordinary room conditions the dehydroascorbic acid comprised about 40 per cent of the vitamin remaining after 24 hours.

The total and reduced ascorbic acid in the leaf lettuce and broccoli, when stored under different conditions, are shown (Tables 3 and 4). Dehydroascorbic acid accounts for a large part of the total vitamin C in lettuce but only a small proportion of the total vitamin C in broccoli. The percentages of oxidized vitamin C in green beans and Swiss chard fall in between the two examples cited. Even though in some cases, e.g., lettuce,

TABLE 2

Comparison of Loss of Ascorbic Acid in Lettuce Stored Normally at Room Temperature and Lettuce Moistened Every Two Hours[1]

Time (hours)	Dry sample			Moistened every two hours		
	Reduced ascorbic acid	Oxidized ascorbic acid	Total ascorbic acid	Reduced ascorbic acid	Oxidized ascorbic acid	Total ascorbic acid
0	23	3	26	23	3	26
6	20	10	30	20	4	24
13	14	10	24	16	5	21
19	11	7	18	14	4	18
24	7	6	13	9	2	11

[1] Units expressed in mgm. per 100 gm.

TABLE 3

Retention of Ascorbic Acid in Leaf Lettuce[1]

Storage	Reduced ascorbic acid	Oxidized ascorbic acid	Total ascorbic acid	Loss
				pct.
Fresh from the farm	27	2	29	....
Packed in crushed ice three days	22	4	26	12
Then in hydrator of mechanical refrigerator three days	7	3	10	66
Then in wire basket of ice refrigerator three days	11	4	15	50
In hydrator of mechanical refrigerator three days	17	3	20	26
In wire basket of ice refrigerator three days	13	6	19	34

[1] Units expressed in mgm. per 100 gm.

TABLE 4

Retention of Ascorbic Acid in Broccoli[1]

Storage	Reduced ascorbic acid	Oxidized ascorbic acid	Total ascorbic acid	Loss
				pct.
Fresh from the farm	140	7	147	....
Packed in crushed ice three days	132	3	135	8
Then in hydrator of mechanical refrigerator three days	107	3	110	26
Then in wire basket of ice refrigerator three days	87	3	90	38
In hydrator of mechanical refrigerator three days	115	0	115	21
In wire basket of ice refrigerator three days	109	0	109	25

[1] Units expressed in mgm. per 100 gm.

the oxidized ascorbic acid makes up a large part of the total vitamin, the percentage loss was approximately the same when reduced or total ascorbic acid was used in calculating percentage loss. In all of these vegetables the loss of total vitamin C was increased by removing the samples from the crushed ice and putting them into the mechanical or ice refrigerator. There was a corresponding increase in the amount of the oxidized vitamin C in the transferred samples.

MECHANICAL AND ICE REFRIGERATOR STORAGE COMPARED WITH ROOM TEMPERATURE

In this study only the reduced ascorbic acid was determined for we were interested in the relative losses and it was necessary to analyze many samples in a limited period of time. In a summary table (Table 5) the values given are the averages of several experiments representing approximately 2,000 individual assays. The following number of experiments was carried out for each vegetable: lettuce, eight; spinach, 10; Swiss chard, four; green beans, five; and broccoli, nine.

TABLE 5

Summary of Percentage Loss of Ascorbic Acid in Vegetables

Time (hours)	Percentage loss of ascorbic acid				
	Room temp.	Mechanical refrigerator		Ice refrigerator	
		Shelf	Hydrator	Shelf	Hydrator
Spinach					
24	70	25	25	50	40
48	70	40	50	55	45
72	....	50	55	55	60
96	....	60	70	60	85
Lettuce					
24	50	25	15	25	25
48	70	40	30	50	35
72	....	45	35	40	30
96	....	65	45	60	55
Broccoli (flowers)					
24	50	10	30	15	15
48	65	10	30	25	20
72	80	30	25	35	20
96	80	40	25	45	30
120	....	40	30	45	45
168	....	....	....	65	60
216	....	....	....	65	60
Green beans					
24	20	10	10	5	5
48	25	10	10	5	5
72	20	10	10	5	5
96	30	10	30	5	5
120	....	30	....	....	....
Swiss chard					
24	35	30	....	....	....
48	65	40	....	....	....
72	....	45	....	....	....
96	85	70	....	....	....

Leaf Lettuce: In studying vitamin retention in lettuce it was of interest to note that the lettuce which had a low reduced vitamin C content when fresh, i.e., four to 12 mgm. per 100 gm., lost this vitamin more rapidly during storage than those samples which had an original value of 12 to 30 mgm. per 100 gm. The former low values are in agreement with the five mgm. per 100 gm. value for leaf lettuce given by Bessey and King (1933). However, most of our ascorbic acid values for fresh leaf lettuce were found in the higher range of 12 to 30 mgm. per 100 gm.

Lettuce stored at room temperature for 24 hours lost approximately 50 per cent of its original ascorbic acid. A sample of the same lot of lettuce which was stored for 48 to 72 hours in the refrigerator, either mechanical or ice, gave an average loss of less than 40 per cent increasing to 60 per cent after 96 hours.

There was no conclusive evidence of superiority of either mechanical or ice refrigeration in the preservation of ascorbic acid. Similarly there seems to be no greater retention of vitamin C when the lettuce was stored in the hydrator pan than when stored on the open shelf.

Spinach: The amount of ascorbic acid in spinach appeared to vary with the maturity of the spinach and with the degree of damage. The spinach, which was obtained directly from the University gardens or from local truck gardens, had a high original vitamin C content of 70 to 90 mgm. per 100 gm. and appeared to lose it more rapidly for the first 24 to 48 hours at room temperature than the samples which were shipped here from California with average values of 30 to 70 mgm. per 100 gm. The differences were 75- and 90-per cent losses for the local samples for 24 and 48 hours, respectively, as compared with 45- and 55-per cent losses in the transported samples. This might be explained on the basis that in this vegetable the greatest destruction of vitamin C takes place just after harvesting and that after the first two days the rate of vitamin C loss is retarded.

Storage in the refrigerators offered some protection for vitamin C. In many cases the spinach could be stored in the ice or mechanical refrigerators for 72 hours with a loss of less than one-half of its vitamin C value. This loss of ascorbic acid appeared to be greater than that reported by Tressler, Mack, and King (1936), for they found almost no loss after three days in the refrigerator. The original value of their spinach, which was grown on upland soil, was reported as 53 to 70 mgm. per 100 gm. This difference might be attributed to a difference in the temperature and humidity in the refrigerator and to the condition of the vegetables stored. The average loss of vitamin C in spinach stored under the conditions specified is shown (Fig. 2).

If the spinach was removed from the hydrator of the refrigerator at 48 and 72 hours and then left at room temperature, the loss of vitamin C was increased to 70 to 80 per cent in 24 hours. This would indicate that refrigeration acted to slow down the rate of oxidation of ascorbic acid, but as soon as the sample was exposed to room temperature the oxidation proceeded at the higher rate.

Green Beans: Green string beans were found to have 10 to 25 mgm. of vitamin C per 100 gm. of vegetables. Contrary to earlier work done in this laboratory and that reported in the literature we found very small losses of ascorbic acid in the green string beans which were stored at room temperature or in the refrigerators. Other workers have reported a 35-per cent loss of vitamin C in beans stored at room temperature for 24 hours and a 57-per cent loss in 48 hours. Mack, Tapley, and King (1939) re-

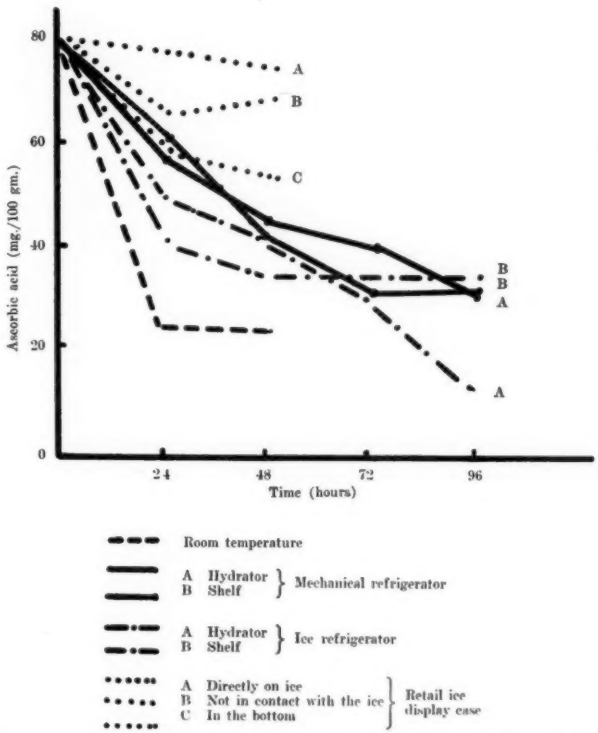

FIG. 2. Average loss of ascorbic acid in spinach when stored as indicated.

ported a loss of 46 per cent after 24 hours and 58 to 78 per cent after six days at room temperature. We found that green beans stored at room temperature for 72 hours, however, lost only about 25 per cent, whereas after 96 hours the loss was 30 per cent. This difference in vitamin C loss may be due to the difference in variety or stage of maturity of the vegetables used.

In a study of the various types of storage within a single mechanical refrigerator we found the hydrator to give the smallest loss of vitamin C after 96 hours, i.e., 10 per cent, while storage in a paper bag on the shelf resulted in a 15-per cent loss and storage on the open shelf gave a loss of

25 per cent. The ice refrigerator was also found to be effective in the preservation of vitamin C; the very small losses of vitamin C when this vegetable was stored in the ice refrigerator, either on the shelf or in the hydrator, are shown (Table 5).

Swiss Chard: Swiss chard was found to be a good source of vitamin C, containing 50 to 95 mgm. per 100 gm. For retention of vitamin C in Swiss chard only a small advantage was gained in storage in refrigerator for the first day. During the first 24 hours the loss at room temperature ranged from 30 to 35 per cent as compared with 20 to 40 per cent loss in the refrigerator-stored samples. However, after 24 hours the chard in the refrigerator seemed to lose the vitamin very slowly, the loss remaining at 40 per cent for three days, while that at room temperature continued to lose vitamin C until after 72 hours the loss was 70 per cent.

Studies on the Swiss chard demonstrated the effect of damage on the loss of ascorbic acid. The damaged chard lost 30 per cent in the first 24 hours, while the undamaged sample lost none when kept at a temperature of 8 to 10°C.(46.4 to 50°F.). If these samples were removed from the refrigerator and left at room temperature for 48 hours, the ascorbic acid was again destroyed more rapidly in the damaged sample, the values being 75 per cent in the damaged and 50 per cent in the undamaged sample. This may be explained on the basis of increased oxidation of the ascorbic acid owing to the increase in surface area exposed and to the liberation of ascorbic acid oxidase from the bruised tissue.

Broccoli: Broccoli stems usually had a lower vitamin C content than the flowers, i.e., 50 to 80 mgm. per 100 gm. of stems, and were found to lose practically no vitamin C when they were stored at room temperature for 48 hours. After 96 hours there was a loss of only 40 per cent.

Broccoli flowers are very rich in ascorbic acid, varying from 75 to 143 mgm. per 100 gm. of tissue. These values are within the range of values reported by Roe (1936); Feener, Palmer, and Fitzgerald (1937); and Wheeler, Tressler, and King (1939). The flowers, however, lost ascorbic acid rapidly at room temperature. The average loss after 24 hours was 48 per cent and increased to 65 per cent after 48 hours. Temperatures of 8 to 10°C. decreased the rate of destruction of vitamin C. The flowers may be stored in a refrigerator, either of the ice or mechanical type, for five days with only 40 per cent loss and approximately 60 per cent loss after nine days.

The broccoli underwent physical changes very slowly in the refrigerator and thus enabled us to continue the experiment for longer periods of time than with any of the other vegetables. The oxidation and consequent destruction of ascorbic acid in this vegetable appeared to have been decreased in rate by the low temperatures of normal refrigeration.

Retail Storage: Normal retail storage of vegetables appeared to have some advantage over laboratory refrigerator storage in the retention of vitamin C. Broccoli left in bunches in the retail mechanical or ice refrigerator for 48 hours gave a 15-per cent loss of reduced vitamin C as compared with a 30-per cent loss in the sample from the same lot taken directly from the shipping crates to the laboratory refrigerator. This difference in favor

of the retail refrigerators may be attributed to the high humidity which they have and to the fact that the vegetables were stored in a more compact manner in the crates and therefore the exposure to oxidation was decreased.

The spinach lost ascorbic acid rapidly at room temperature, 45 per cent in 24 hours, and fairly rapidly, 35 per cent, in the top of the ice display case when it was not in contact with the ice (Table 6). However, storing the vegetables directly on the ice reduced the loss of vitamin C to approximately five per cent. Broccoli demonstrated a similar range in the retention of vitamin C. The effect of storage in the bottom of the ice

TABLE 6

Comparison of Spinach and Broccoli Stored in a Retail Ice Display Case With That Stored at Room Temperature

Time (hours)	Percentage loss of ascorbic acid			
	Room temp.	Top of ice display case		Bottom of display case
		Directly on ice	Not directly on ice	
Spinach				
10	35	5	25	15
24	45	5	35	15
48	65	....	....	....
Broccoli				
10	15	....	5	0-2
24	50	....	20	0
48	70	....	....	....

display case varied with the vegetable. Broccoli lost 0 to two per cent of the original vitamin C value, while spinach lost 15 per cent in 24 hours. The samples stored directly on the ice had the advantage of low temperatures, high humidity, and decreased exposure to air movements.

DISCUSSION

All of the vegetables studied lost ascorbic acid at a rapid rate after being harvested if measures were not taken to prevent this loss. There was considerable variation in the rate of loss and in the form of vitamin C in the different vegetables when held under similar conditions of storage. The vitamin was most labile in the lettuce and spinach and more stable in broccoli, green beans, and Swiss chard. Similarly lettuce had a greater amount of its vitamin C in oxidized form than the other vegetables, with broccoli having the smallest proportion of dehydroascorbic acid present.

That dehydroascorbic acid is of importance in ascorbic acid studies and that the quantity of this form present varies with the vegetable and the period of storage has been definitely established. If relative rather than actual values are desired, however, studies on the reduced ascorbic acid alone were found to give reliable results.

Moisture without refrigeration is of no benefit in the preservation of vitamin C. Lettuce moistened at room temperature showed just as great a loss of vitamin C as that which was allowed to desiccate. Refrigeration reduced the loss of vitamin C in all cases but refrigeration alone is not the

complete answer. The loss was reduced to 12 per cent in the green beans and approximately 30 per cent in the broccoli but the spinach lost as much as 55 per cent in three days in the refrigerator.

Packing of the vegetables in the crushed ice is a practical and effective means of preventing deterioration of these vegetables during transportation and storage. This type of storage combines the factors of humidity and low temperature. Vegetables so packed retain their desirable qualities of color, weight, and freshness. The loss of total vitamin C in the ice-stored samples also varies with the kind of vegetables. Roughly there is no loss in the case of Swiss chard, 10 per cent in broccoli, 20 per cent in lettuce, and 30 per cent in green beans over a period of three days. It should be emphasized that proper conditions of both humidity and temperature, starting at the time the vegetables are harvested, should be maintained. Retail-store ice or mechanical refrigerators appeared to provide better conditions for the protection of vitamin C than the smaller household models. However, the retail ice display cases seemed to offer the best protection for the ascorbic acid in most vegetables when the vegetables were placed directly on the ice.

CONCLUSIONS

Ascorbic acid is destroyed quite rapidly in spinach, chard, lettuce, and broccoli at room temperature of 20 to 23°C. (68 to 73.4°F.).

Packing the vegetables in crushed ice as soon as they were harvested in the field was found to be an effective means of preventing the destruction of vitamin C during transportation and storage.

Storage of these vegetables in either the mechanical or ice refrigerators had a marked and favorable effect on the retention of ascorbic acid.

Retail storage of these vegetables in crates in the refrigerators was characterized by a slow rate of destruction of ascorbic acid.

The factors involved in the retention or loss of ascorbic acid may include the physical condition of the vegetables, humidity and temperature of the storage, and exposure to oxidation.

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GROWTH OF A FOOD-POISONING STRAIN OF ALPHA-TYPE *STREPTOCOCCUS* EXPERIMENTALLY INOCULATED INTO CANNED FOODS[1]

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Outbreaks of food poisoning due to alpha-type streptococci are not well recognized. Nevertheless, a small number of these outbreaks has been reported by Dack (1943). Although alpha-type streptococci are killed by the time and temperature used in processing canned foods, the ability of these organisms to grow and survive in such products is of practical importance to the problem of food poisoning. Contamination of the contents of a can with these organisms may occur after the can is opened, following which storage at temperatures supporting growth might lead to the development of large numbers of organisms capable of giving rise to gastrointestinal symptoms on ingestion.

The purpose of the present investigation, therefore, was to study the growth of a food-poisoning strain of alpha-type *Streptococcus* in experimentally inoculated cans of food. By this method pure cultures could be studied, and the possibility of involvement of canned foods in this type of food poisoning elucidated. Previously, similar studies have been carried out using *Salmonella enteritidis*, Segalove and Dack (1944), and a food-poisoning strain of *Staphylococcus aureus*, Segalove, Davison, and Dack (1943).

Canned foods studied in these experiments include asparagus, spinach, string beans, shrimp, salmon, peaches, tomato juice, peas, and corn. These foods represent a cross section of several different types of canned food on the market; for example, peas and corn are low-acid products; asparagus, spinach, and string beans are semiacid products; tomato juice and peaches are acid products. Shrimp and salmon were included as examples of sea food, the chemical composition of which is markedly different from fruits and vegetables.

EXPERIMENTAL PROCEDURE

In all experiments a food-poisoning strain of alpha-type *Streptococcus* was used. This strain was isolated from turkey dressing in 1939 and has been kept on blood agar medium since that time. The organisms were grown in veal infusion broth for 18 to 24 hours and the broth culture was inoculated directly into the various cans of food. The technic of handling the cans has been previously described by Segalove, Davison, and Dack (1943). The total number of organisms inoculated per can of food was determined by plate counts. These varied from 105×10^5 and 188×10^7 organisms per can. The number of organisms per milliliter of contents of the can was calculated by dividing the total count in the inoculum by

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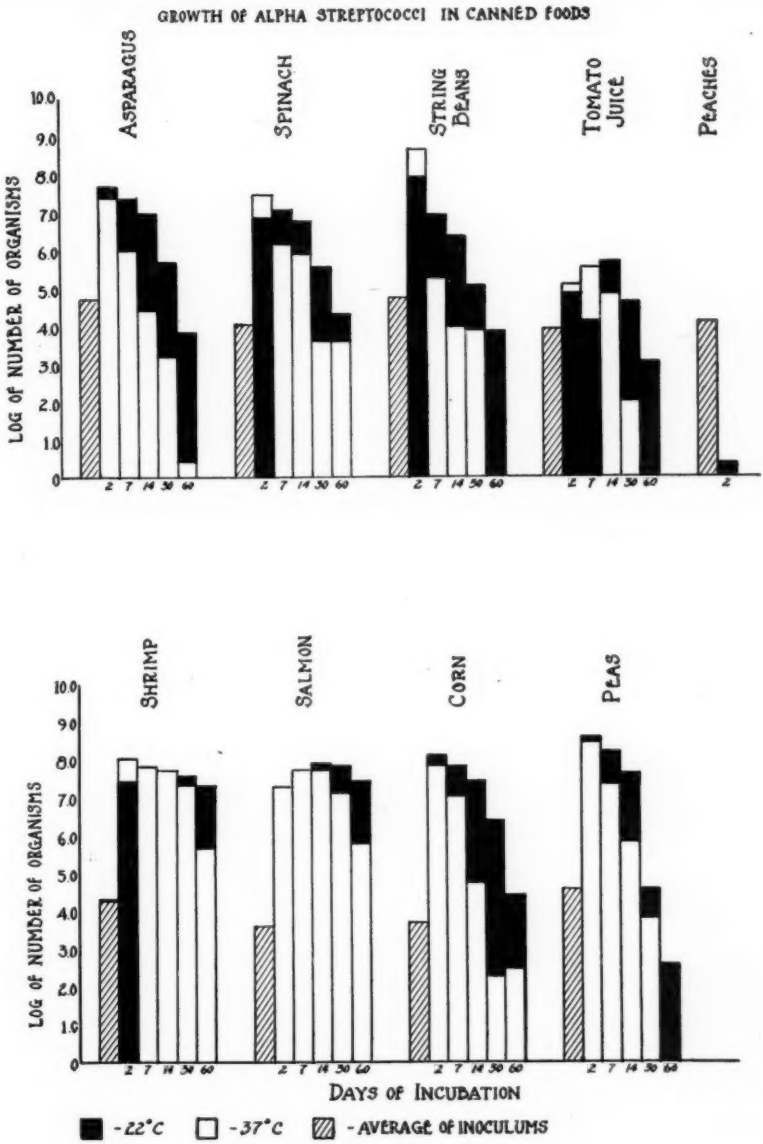
GROWTH OF ALPHA STREPTOCOCCI IN CANNED FOODS
ASPARAGUS
SPINACH
STRING BEANS
TOMATO JUICE
PEACHES
SHRIMP
SALMON
CORN
PEAS
LOG OF NUMBER OF ORGANISMS
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
2 7 14 30 60
2
DAYS OF INCUBATION
-22°C
-37°C
-AVERAGE OF INOCULUMS

FIG. 1.

the total number of milliliters capacity of the can. The total inoculums in each experiment were the same for each type of food. Eight cans of each food except peaches and tomato juice were always inoculated. Twelve cans of peaches and tomato juice were inoculated. Half of the total number of cans of each food studied were incubated at 37°C.(98.6°F.) and the other half at 22°C.(71.6°F.) for varying periods of time.

At intervals of 2, 7, 14, 30, and 60 days, cans were removed from the incubators, shaken, and samples removed for count. Decimal dilutions of the samples were made in sterile 0.85 per cent NaCl solution and blood agar pour plates were made with one-ml. portions of the decimal dilutions. After 48 hours' incubation at 37°C. the colonies on each plate were counted.

RESULTS AND DISCUSSION

The results are shown in a graph (Fig. 1), in which the ordinate represents the logarithm of the arithmetical average of bacterial counts per milliliter of fluid contents of the can at 37 and 22°C. Several points are clear from the results obtained. No growth occurred in canned peaches, a highly acid product, and organisms were not demonstrable at either temperature after two days. Attention is called to the fact that the peaches were preserved in syrup, an added factor in bacteriostasis. In tomato juice, the other highly acid product, growth occurred slowly, the highest counts being obtained after seven days at 37°C. and after 14 days at 22°C. Organisms were demonstrable in all foods except peaches for 30 days at both 22 and 37°C. and for 60 days at 22°C. In many instances higher counts were obtained at the latter temperature, a finding of some significance in regard to possible involvement of these foods in food poisoning. The survival of organisms was not different in the case of semiacid foods (asparagus, spinach, string beans) and low-acid foods (corn, peas). Insignificant differences in counts from cans incubated at 37 and 22°C. were found in the case of sea-food products. Survival of organisms in salmon and shrimp was significantly better than in the fruits and vegetables.

SUMMARY

The growth and survival of a food-poisoning strain of alpha-type *Streptococcus* has been demonstrated for at least 30 days at 37°C. in canned foods of low-acid content (corn, peas), semiacid content (asparagus, spinach, string beans), a highly acid product (tomato juice), and in sea-food products (shrimp and salmon). Organisms were demonstrable in all cases for 60 days at 22°C. and at 37°C. in most instances. Growth did not occur in canned peaches, sugar preserved (a highly acid product), and viable organisms were not cultured after two days.

The significance of these findings to the problem of food poisoning has been discussed.

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BACTERIOLOGICAL STUDIES OF REFINED SUGARS ON THE RETAIL MARKET

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Stimulated interest in the home canning of vegetables, fruits, and meats raises serious questions. Does the sugar acquired on the retail market by the housewife meet the standards laid down by the National Canners Association (1940) to protect its members against loss by sugar-borne spoilage organisms? Is the housewife forced to use contaminated sugar?

In order to prevent the spoilage of canned foods much work has been done on the bacterial flora of sugar used in commercial canning. Sugar used for home canning should meet the same standards as that used in commercial canning. While figures on the yearly production of home-canned goods are not available, there must be, especially during these war years, a large quantity of such foods. Only comparatively small lots of home-canned foods may be spoiled on any single occasion, but the country-wide damage may reach an impressive total. The experiments here reported were designed to determine whether sugar sold for home consumption meets the standards set up for that used in commercial canning.

In the contamination of sugar and the consequent spoilage of nonacid foods the most important bacteria are the sporeforming thermophiles, chiefly the facultatively anaerobic "flat sours" [*Bacillus stearothermophilus*, Donk (1920)] the obligately anaerobic, hydrogen sulfide-producing "sulfur stinkers" [*Clostridium nigrificans*, Werkman and Weaver (1927)]; and the anaerobic "hard swell" type [*Clostridium thermosaccharolyticum*, McClung (1935b)]. Mesophilic bacteria are less important spoilage organisms.

Thermophilic spoilage bacteria were first found in refined sugar in 1926, according to Cameron and Yesair (1931). Owen and Mobley (1932) demonstrated that thermophiles entered cane sugar with raw sugar, dust, and bag sizing. Calton (1936) showed that the "flat-sour" thermophiles entered beet sugar with cossettes (shredded beets) and house water and developed on walls and in liquor in pan storage tanks.

During the season of 1926, Cameron and Williams (1928) noted that sugar was an important vehicle in thermophilic contamination in corn canneries. They found "flat sours" in practically all the cane-sugar samples examined and in numbers as high as 1,000 per 10 grams. Obligately anaerobic thermophiles were rarely found. In beet sugar "flat sours" were not often present, while thermophilic obligate anaerobes were found in larger numbers than in cane sugar; however, no hydrogen sulfide-

producing anaerobes were found. Cameron, Williams, and Thompson (1927) stated that sugar was the only definite external source of thermophilic contamination in canneries processing corn and peas. Cameron and Yesair (1931) found several canners of corn and peas using sugar from one source that contained all three types of spoilage thermophiles. "Flat sours" ran as high as 2,000 per 10 grams. These resisted 115°C.(239°F.) for 145 to 150 minutes in dilute pea purée, and for 70 to 80 minutes in the liquid from canned corn.

McClung (1935a) stated that Homans found the recognized groups of thermophiles in Belgian, Czechoslovakian, and Dutch sugars. Clark and Tanner (1937) showed the three types of thermophiles to be present in practically all of 91 samples of beet and cane sugar examined. Twenty-five of these samples were secured from a corn cannery.

The establishment of bacterial standards for sugar was the outgrowth of this work on the relationship between the bacteriological condition of sugar and thermophilic spoilage of nonacid canned products. The bacteriologists of the National Canners Association (N.C.A.) have done much work on this problem, and in 1931 published their first methods and standards for judging sugar used by canners; these were revised in 1932 and again in 1933 (N.C.A. 1940). Their standards (for individual samples) follow:

> Total thermophilic spores—not more than 150 spores per 10 grams.
> Flat-sour spores—not more than 75 spores per 10 grams
> Sulfide-spoilage spores—not more than 5 spores per 10 grams
> Anaerobic spores—no H_2S—present in not more than 4 of the 6 tubes containing a total of 4 grams sugar.

Owen and Mobley (1935) suggested that bacterial contamination of sugar, other than by thermophiles, deserved more study. The numbers of mesophiles, yeasts, and molds remained an unknown quantity, although such organisms might cause serious difficulties in soft-drink plants and other non-sterilizing industries. Only bacteria survive the temperature used in manufacturing sugar; yeasts and molds gain access at the centrifugals or on the conveyors. Owen recommended tentative standards for mesophilic bacteria, yeasts, and molds in granulated sugar. He suggested a maximum of 50 mesophiles per gram of sugar.

In a report to the N.C.A. Cameron and Esty (1941) listed the following results of the testing of sugar by N.C.A. standards—1936, 93.1 per cent of 564 samples passed; 1937, 94.6 per cent of 599 samples passed; 1938, 86.6 per cent of 725 samples passed; 1939, 89.2 per cent of 897 samples passed; 1940, 94.1 per cent of 1,008 samples passed.

EXPERIMENTAL PROCEDURE

The sugar tested in this work was purchased at retail stores and markets. Most samples were of the (commonly used) berry granulated sugar, but a few packages of fine granulated (bar and dessert) and raw cane sugar were bought. Both beet and cane sugar, packed in paper and cloth bags and in cartons, were tested. The paper bags were filled at the

stores or, in the case of one large chain of stores, at the central warehouse; the cloth bags and cartons were factory packed. To get as thorough a survey as possible, only one- and two-pound packages were secured (not more than one of a kind in each store). Numerous samples were collected on trips around Los Angeles; each trip was made to a different neighborhood to reduce the possibility of duplication. The samples were numbered and the number, kind of sugar, and type of package were entered on a chart.

From each sample 20 grams were aseptically weighed into a 250-c.c. Erlenmeyer flask. Sterile water was added to make 100 c.c. The sugar was completely dissolved by agitating and slightly warming the flask. For the mesophilic aerobes duplicate Petri plates of Standard Methods nutrient agar were inoculated with two c.c. (0.4 gram of sugar) each of the sugar solution. Dextrose agar shake cultures were inoculated in duplicate with five c.c. (one gram of sugar) each of the sugar solution for the determination of anaerobic counts. These plates and tubes were incubated at 37°C. (98.6°F.) and counted after 48 hours. After these inoculations the sugar solutions were boiled for five minutes to destroy the non-sporulating organisms present. Sterile water was added to each flask to replace the water which had boiled away. Twenty c.c. (four grams of sugar) of each solution were then divided equally among six tubes of melted sulfite agar, Cameron (1938). The tubes were rolled as shake cultures. This medium is used to detect the presence of thermophilic anaerobes producing hydrogen sulfide. For other thermophilic anaerobes, 20 c.c. of the solutions were divided equally among six tubes of liver broth, Cameron (1936a,b). This medium was heated and cooled immediately before using to render it anaerobic. After inoculation and further cooling the medium was stratified with approximately 1.5 inches of sterile agar (1.5 per cent). An attempt was made to substitute for this liver broth the corn-liver medium described by McClung and McCoy (1934). A number of the sugar samples were inoculated into the corn-liver as well as the liver broth for comparison. The results with the corn-liver medium were not satisfactory. The quantity of inoculum used caused what appeared to be a hydrolysis at the top of the medium; actually it was merely the inoculum, which had risen to the top. Inoculation of an equal amount of sterile water gave the same apparent hydrolysis. In some cases small bubbles were seen along the sides of the tubes after 24 hours' incubation, but the amount of gas was so small that it was difficult to tell whether it was produced by bacteria or was merely trapped air. This corn-liver medium is inexpensive and requires no seal for anaerobes, but it must be carefully prepared and autoclaved and is apparently not suited for the examination of sugar.

For the detection of facultatively anaerobic thermophiles, including "flat sours," the tryptone medium described by Williams (1936), Cameron (1936a,b), and the N.C.A. (1940) was used. Two c.c. of the sugar solutions were placed in each of five plates, using 10 c.c. in all. The inoculated sulfite agar, liver broth, and tryptone medium were incubated at 55°C.(131°F.) for 72 hours, at the end of which time counts were made.

TABLE 1

Facultative and Aerobic Bacteria in Refined Retail Sugars

Bacteria		Type of sugar and container							Total
		Beet, paper bag	Beet, cloth bag	Cane, paper bag	Cane, cloth bag	Cane, carton	Cane (fine), carton	Cane (raw), carton	
Number of samples tested		38	4	24	38	36	8	2	150
Thermophilic sporeformers:	Positive samples	23	2	16	20	14	0	0	75
Aerobes and facultative anaerobes	Substandard samples	1	0	3	1	0	0	0	5
	Av. bacteria per 10 gm	13.9	7.5	72.9	11.7	6.7	0	0	19.9
	Positive samples	23	2	15	14	12	0	0	66
Flat sours only	Substandard samples	1	0	5	1	0	0	0	7
	Av. bacteria per 10 gm	11.1	7.5	62.5	9.4	3.1	0	0	16.1
	Positive samples	38	4	24	37	34	8	2	147
Mesophiles	Substandard samples	1	0	1	0	0	0	0	2
	Av. bacteria per 10 gm	111.0	128.0	77.6	79.0	34.0	19.7	16.0	65.2

Controls with known organisms were run to show that the media were not faulty.

RESULTS

The results were grouped according to the kind of sugar and manner of packaging and are summarized (Table 1). Aerobes or facultatively anaerobic thermophiles, including "flat sours," were present in 50 per cent, or 75 of the 150 samples tested. Not shown in Table 1 are the hydrogen sulfide-producing anaerobes, which occured in only two, or 1.33 per cent, of the samples, and other anaerobic thermophiles present in four, or 2.66 per cent, of the samples. Seven samples exceeded the standards of the National Canners Association; of these, six were cane and one was beet sugar. Two samples did not meet Owen's suggested standard for mesophiles; both of these were from paper-bagged sugar. The average count on sugar in cardboard cartons was much lower than on that in paper and cloth bags. Paper bags are usually filled by hand and are subject to contamination. The two-pound cloth bags offer a poor barrier to the entrance of bacteria. A more detailed analysis of these bacterial counts is given by Wolk (1941).

A comparison of these results with those of previous workers, who tested cannery grade sugar, will show that the bacteriological quality of refined sugar has improved.

SUMMARY

Using the methods of the National Canners Association 150 samples of beet and cane sugar, packed in one- and two-pound paper, cloth, and cardboard containers, were tested for thermophilic bacteria. In addition counts were made of mesophilic bacteria. The sugar was purchased at random in retail stores in Los Angeles; presumably no two samples were from the same lot or shipment, but there is no definite assurance of this fact. The results are summarized (Tables 2 and 3).

TABLE 2

Bacteriological Quality of 150 Samples of Retail Sugar Selected at Random

	Samples	Percentage
Passing N.C.A. standards	143	95.33
Passing Owen's suggested standard	148	98.66

TABLE 3

Occurrence of Various Spoilage Thermophiles in 150 Samples of Retail Sugar Selected at Random

	Samples	Percentage
All aerobes and facultative anaerobes	75	50.00
Flat-sour, facultative anaerobes	66	44.00
Non-flat-sour facultative anaerobes	26	17.33
H_2S producing, obligate anaerobes	2	1.33
Non-H_2S producing, obligate anaerobes	4	2.66

CONCLUSIONS

Sugar sold at retail is of as high quality, bacteriologically, as that used in commercial canning of nonacid foods. The housewife is afforded great protection against sugar-borne spoilage bacteria. The use of cardboard cartons seems to offer more protection from contamination than do other methods of packaging.

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RIBOFLAVIN CONTENT OF IMMATURE MASSACHUSETTS LETTUCE [1]

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Many of the riboflavin-rich natural foods, such as milk, cheese, and the glandular meat products, are either rationed or likely to be in the near future. Consequently, in the future greater reliance must be placed upon vegetable foods which can supplement the restricted supply of riboflavin-rich animal foods. Since lettuce is a readily available and commonly used leafy vegetable, its riboflavin content is of particular interest. A review of the literature, however, revealed rather limited data concerning the riboflavin content of lettuce.

Poe and Hartman (1940) concluded from feeding Colorado head lettuce to rats that both the outer and inner leaves contained 55 to 67 Sherman units of riboflavin per 100 grams. Cheldelin and Williams (1942) found the riboflavin content of lettuce to be 27 micrograms per 100 grams. Munsell (1942) studied lettuce by the rat-growth method and concluded that it contained less than 0.25 microgram of riboflavin per gram. Hodson (1940) found 0.48 microgram of riboflavin per gram for head lettuce, 0.96 for stemmy lettuce, and 0.71 for leaf lettuce. Wilson and Roy (1938) report that lettuce grown in India contained 116 micrograms of riboflavin per 100 grams. Flavier and Genevois (1939) found from 60 to 80 micrograms of riboflavin in the inner leaves and 30 to 80 micrograms per 100 grams in the outer leaves of lettuce.

Several of the investigators stated that the lettuce studied was purchased in local markets. Thus the reported results may indicate the riboflavin value of lettuce obtained from stores, but it is questionable whether or not they supply accurate information concerning the riboflavin content of lettuce that the farmer and home gardener consume shortly after it is removed from the ground. Since the above-mentioned authors studied lettuce grown in America, France, and India, it is possible that the lettuce under investigation was produced under a wide variety of conditions. In this connection it may be noted that Holmes and Tripp (1933) as well as numerous investigators cited by them, called attention to the pronounced effect of soil, fertilizer, and climatic conditions upon the chemical composition of plants. In view of the limited amount of information available and the lack of agreement of data reported in the literature it seemed desirable to accumulate additional data concerning the riboflavin content of lettuce.

EXPERIMENTAL PROCEDURE

The lettuce used in this study was grown on the College Farm under conditions quite typical for this locality. According to Emerson (1897)

[1] Contribution No. 492, Massachusetts Agricultural Experiment Station.

the area upon which the lettuce was grown is the bottom of the Hadley Postglacial lake. The alluvial soil is supposed to be a modified drift and, in the opinion of Gordon (1943),[2] accumulated during a late stage in the waning of the ice sheet. Beaumont (1943a,b) states that it is glacial outwash material classified as a Merrimac fine sandy loam. Two feet or so below the surface is a layer of gravel which provides satisfactory drainage of the top soil at all times.

For the past five years the soil had been fertilized with a special mixture of sodium nitrate, ammonium sulfate, ammonium phosphate, and potassium chloride applied at the rate of one ton per acre and with 1,000 pounds of limestone per acre. Each of the first three ingredients furnished one-third of the nitrogen. The limestone supplied calcium and neutralized the soil acidity. The soil upon which the lettuce was grown had a hydrogen-ion value of pH 4.4.

The climatic conditions for the six weeks or more that the lettuce was growing are reported by Gunness (1943) as follows: rainfall for May, 5.62 inches and for June, 2.38 inches; temperature (Fahrenheit) for May, maximum 84°, minimum 31°, average 57.8° and for June, maximum, 95°, minimum 48°, average 71.1°; bright sunshine for May, 374 hours and for June, 356 hours. These climatic conditions were quite similar to normal except that the normal rainfall for May is 3.6 inches.

Boston head lettuce was used for this study. The seed was sown in the greenhouse on April first and the young plants were transplanted in the field on May first.

Ordinarily this type of lettuce is not marketed until it has formed compact heads and then the outer leaves are largely removed. In this study the immature lettuce was picked during a three-week period, just before it began to form heads. At this stage all the leaves were spread out and exposed to light and sunshine, a condition quite different from that existing when the lettuce has developed fully formed heads. The plants were pulled from the ground early in the morning and taken to the laboratory where they were washed free from any soil. The entire leafy portion of each plant except the lower part of the midribs of the leaves was used. The individual leaves were dried by patting gently with cheesecloth. The leaves were then piled one upon another and tightly rolled to produce a cylinder that could just be held in the hand. Small particles were cut with shears from the cylinder directly into the extraction flask. Crisp, freshly picked lettuce wilts rapidly when finely cut but in this instance dehydration was eliminated, for the lettuce was in the extraction flask within 10 minutes after it reached the laboratory and within 20 minutes after it was pulled from the ground.

The riboflavin was released from the lettuce by acid extraction. About 50 grams of lettuce were extracted with 100 c.c. of 0.25 N sulphuric acid on a hot plate under a reflux condenser until the particles of leaves were disintegrated. The length of the extraction period varied according to conditions. If particles of lettuce tended to remain on the surface of the

[2] Gordon, Clarence E., Professor of Geology and Mineralogy, Massachusetts State College. Personal communication.

boiling extraction liquid in spite of vigorous shaking of the flask, the extraction period was lengthened. In general, the extraction period was about three hours but the particles of lettuce leaves were reduced to pulp in much less time.

The extraction mixture was cooled, treated with sufficient 6.5 per cent trisodium phosphate solution to produce a pH of 6.8, and filtered. A 100-c.c. aliquot was placed in a 200-c.c. volumetric flask. Two cubic centimeters of stannous chloride (prepared by dissolving 10 gm. of stannous chloride in 25 c.c. of concentrated hydrochloric acid and diluting one c.c. of the mixture to 200 c.c. with distilled water) and two c.c. of sodium hyposulphite solution (prepared by dissolving one gm. of $Na_2S_2O_4 \cdot 2H_2O$ and one gm. of $NaHCO_3$ in 20 c.c. of ice-cold distilled water) were added to reduce all the materials which produce fluorescence. The mixture was

TABLE 1

Riboflavin Content of Immature Massachusetts Lettuce

Sample No.	Weight	Extracted	Riboflavin	Sample No.	Weight	Extracted	Riboflavin
	gm.	*hr.*	*mgm./ 100 gm.*		*gm.*	*hr.*	*mgm./ 100 gm.*
1	27.5	1.0	.155	10	62.1	3.0	.105
2	41.6	2.0	.113	11	54.9	3.0	.126
3	34.9	2.0	.139	12	47.5	3.3	.113
4	63.5	3.0	.150	13	49.8	3.3	.114
5	63.4	3.0	.109	14	44.3	3.3	.122
6	63.2	2.5	.130	15	54.8	3.2	.125
7	61.9	2.5	.131	16	60.4	3.2	.105
8	59.2	3.0	.135	17	60.4	3.2	.127
9	64.9	3.0	.118				Av. .124

made up to 200 c.c. and the riboflavin was reoxidized. Subsequently three 50-c.c. aliquots were used for the riboflavin determination. One aliquot "C" was reduced with one c.c. of the sodium hyposulphite solution and served as a negative control. One c.c. of standard riboflavin solution was added to the second aliquot "B" and this served as a positive control. One c.c. of distilled water was added to the third aliquot "A" to produce equal volumes for all three aliquots. Ten c.c. of each aliquot were transferred to cuvettes for fluorometric assay.[3] By reading the negative control "C" both before and after reading sample "A" and aliquot "B," one can detect and check any change in electric current, setting of the instrument, or other mechanical factors which would affect the accuracy of the reading of the sample; but such constant checking of the instrument is not possible when one relies upon a reference curve for interpreting the reading of the samples.

The values of "A," "B," and "C" supply data for computing the riboflavin content of sample "A." Throughout the analytical procedure every precaution was taken to prevent destruction of riboflavin. Since riboflavin may be destroyed by light [Hogan and Hunter (1928); Supplee, Ansbacker, and Bender (1935); György (1935); and others], amber

[3] Coleman Universal spectrophotometer fluorometer.

glassware was used and the assays were made in a specially prepared semibasement laboratory. All daylight was excluded by light-impervious paper, and the 100-watt Mazda bulbs were surrounded with long metal cylinders that prevented direct light reaching the laboratory tables at the side of the room. Light-meter measurements of the light intensity at the site of the precipitation, filtration, and other assay procedures showed from one- to two-foot candle power.

RESULTS

The results of riboflavin assays of 17 samples (Table 1) show that the riboflavin content of lettuce varied from 0.105 to 0.155 mgm. and averaged 0.124 mgm. per 100 gm. While an attempt was made to use lettuce plants of the same size and stage of maturity, it is quite evident that plants of immature lettuce differ significantly in their riboflavin content. Consequently, many more data will be required before the variation in the riboflavin content of lettuce grown under the conditions described can be correlated with any of the factors which influence the rate of growth or the composition of the lettuce. This value, 0.124 mgm. of riboflavin per 100 gm., is much higher than the values reported by the investigators cited above. It is possible that this higher value is partly due to the fact that, in this study, the lettuce was assayed within a very short time after it was removed from the ground, whereas that studied by other investigators was obtained from local markets and probably was harvested hours and possibly days earlier. The higher value reported here may be due also to the immature stage of development of the lettuce, whereas that discussed by others was doubtless fully grown.

It is interesting to compare the value, 0.124 mgm. of riboflavin per 100 gm. of lettuce, with the riboflavin content of milk, a food which is quite generally recommended as an excellent source of riboflavin. Holmes and Holmes (1943) found that December, January, February, and March milk contained 1.45, 1.50, 1.49, and 1.46 mgm. of riboflavin per liter; and Holmes, Jones, Wertz, and Kuzmeski (1943) reported that raw milk contained 1.51 $\pm$ 0.09 mgm. of riboflavin per liter and corresponding pasteurized milk, 1.48 $\pm$ 0.01 mgm. The cows producing the milk were fed forage crops grown on soil similar to that which produced the lettuce under discussion. Hence, a comparison of the riboflavin content of the lettuce and milk in the present instance may have greater significance than a comparison made between the riboflavin content of the lettuce under investigation and that of milk produced in different localities. When the value for milk is converted to the same basis as that of lettuce, the comparison becomes 0.150 mgm. of riboflavin per 100 gm. of milk and 0.124 mgm. of riboflavin per 100 gm. of lettuce; in other words, the immature Boston head lettuce used in this study contained more than 80 per cent as much riboflavin as the milk produced on the same farm. From a practical standpoint this comparison is applicable only to a limited extent because it is impossible for a person to conveniently consume as large an amount of lettuce daily as he ordinarily could consume of milk. However, it is quite evident from the results of this study that immature Boston head lettuce grown under the conditions described is an excellent vegetable

source of riboflavin for supplementing the decreasing amount of foods of animal origin.

SUMMARY

Since the available supply of riboflavin-rich foods of animal origin is being curtailed, it is desirable to assess the riboflavin value of common vegetables. Lettuce is reported to be relatively rich in riboflavin but the literature consulted contained limited data which indicated market lettuce contains from 27 to 116 micrograms of riboflavin per 100 gm. In this study immature Boston head lettuce grown under known soil, fertilizer, and climatic conditions was assayed. Seventeen samples gave an average value of 0.124 mgm. per 100 gm. Thus this immature lettuce contained over 80 per cent as much riboflavin as milk produced by cows that consumed forage crops grown on similar soil. Obviously lettuce cannot be consumed in as large amounts as milk, but immature lettuce of this type should serve as a valuable supplement for the decreasing riboflavin-rich animal foods available for human dietary.

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EFFECT OF VARIOUS TEMPERATURES OF STORAGE UPON CHEMICAL AND PHYSICAL PROPERTIES OF POWDERED EGG YOLK AS RELATED TO ITS USE IN ICE CREAM

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The utilization of powdered-egg products in food industries has greatly increased during recent years. Dried whole eggs, because of their importance in the human dietary and because of their convenient form for shipping to distant markets, have an important place in the war emergency program. In normal times, dried egg yolk has been extensively used by commercial ice cream manufacturers because of its beneficial effect upon mix whipping and the color and flavor of the finished product.

The literature contains little information regarding the keeping quality of either powdered whole egg or egg yolk. That powdered whole milk deteriorates upon storage especially at high temperatures has been pointed out by Hollender and Tracy (1942). Since egg yolk contains a high fat percentage, it is to be expected that certain changes will also occur in the chemical and physical properties of both dried egg yolk and whole egg during storage. Such information could be of importance to commercial food manufacturers who find it desirable to purchase their dried-egg supplies months in advance.

The primary purpose of this investigation was to determine the effect of various storage temperatures upon the keeping quality of powdered egg yolk as related to its use in commercial ice cream manufacture.

EXPERIMENTAL PROCEDURE

Four samples of spray-dried, powdered egg yolk were used. Sample A was manufactured in Missouri during the month of July. Sample B was produced in Texas, Sample C was made from New Jersey eggs, and Sample D came from Nebraska. The last three samples were produced during the months of May and June, 1941.

When received, the powdered eggs were analyzed for bacteria and total solids. The eggs were then packed into sterilized, one-pound, sanitary, enameled tin cans and sealed at atmospheric pressure. Samples of each powder were stored at temperatures of —20.6 to —28.9, 4.4, 21.1, and 32.2°C. (—5 to —20, 40, 70, and 90°F.). A sample of the powdered eggs was then analyzed for the following factors: percentages of fat, ash and protein, pH, and solubility. The percentage of fat, ash, and protein were determined by the methods described in the *Official and Tentative Methods of Analysis* of the Association of Official Agricultural Chemists. The pH was determined on a 10-per cent solution of egg in previously boiled and cooled distilled water, using a Beckman potentiometer. The milliliters of

[1] On military leave.

insoluble material were determined by the method described for determining the solubility index of milk powders by the American Dry Milk Institute.[2] The complete analyses of the four samples before storage are given (Table 1).

TABLE 1

Composition of Four Samples of Powdered Egg Yolk Before Storage

Sample	pH	Protein	Fat	Total solids	Ash	Total bacteria per gram	*Escherichia coli* per gram	Insoluble material	Flavor
		pct.	*pct.*	*pct.*	*pct.*			*ml.*	
A	6.50	32.30	54.30	95.55	2.47	420	110	4.5	Very good
B	6.45	33.50	52.37	95.94	2.72	4,000	1,300	7.0	Good
C	6.20	32.42	54.80	97.20	2.81	800	10	8.0	Good
D	6.95	41.50	35.45	94.00	3.97	140	50	5.0	Excellent

Samples A, B, and C were approximately of the same composition. Sample D, which was a mixture of egg yolk powder and dried skim milk, contained a higher protein and ash content and a lower fat content than the other samples. It was noted that this sample was heavily seeded with mold.

All samples of powdered egg yolk were judged for flavor, odor, and color. The powder was then used as a constituent of ice cream mix containing 14 per cent fat, 10 per cent serum solids, 0.4 per cent egg powder, 0.35 per cent gelatin, and 15 per cent sugar. A control mix was also made using exactly the same products excepting the egg powder. The mixes were pasteurized at 71.1°C.(160°F.) for 30 minutes, homogenized at 2,000 pounds pressure using a single-stage type of homogenizer, and then cooled to 4.4°C.(40°F.). Basic-mix viscosity determinations were obtained after 24 hours, using a 50-milliliter pipette. The mixes were frozen on a counter freezer.

Temperature and overrun determinations were made at one-minute intervals. All ice cream samples were drawn at approximately —3.9°C. (25°F.) and 100 per cent overrun. After 24 to 48 hours the ice cream was judged for body, texture, flavor, and melt-down characteristics.

Periodically during a period of 18 months, sets of the powdered-egg samples were analyzed for pH, solubility, total bacteria per gram, flavor, odor, and color. Ice cream mixes were made with each powder stored at the various temperatures.

EXPERIMENTAL RESULTS

Analysis of the samples of egg powder as determined periodically during the 18 months' experimental period are given (Table 2).

The temperature at which powdered egg yolk is stored was found to be very important. At room temperature or above the powder underwent a rather rapid change as indicated by a marked decrease in pH and increase in amount of insoluble material present. Color became less golden, chang-

[2] American Dry Milk Institute Inc., Chicago, Illinois. The Grading of Dry Milk Solids.

TABLE 2

Analysis of Four Samples of Powdered Egg Yolk After Periods of Storage at Different Temperatures

Storage time	pH	Insoluble material (ml.)	Total bacteria per gram	Flavor	Color
SAMPLE A					
(Stored at —5 to —20°F.)					
At storage	6.50	4.5	420	Very good	Golden yellow
After 6 weeks	6.50	4.5	400	Good	Golden yellow
After 12 weeks	6.28	7.62	600	Good	Golden yellow
After 24 weeks	6.02	7.6	700	Good	Golden yellow
After 36 weeks	6.15	9.0	1,000	Good	Golden yellow
After 52 weeks	5.79	7.5	2,100	Good	Golden yellow
After 72 weeks	6.31	8.0	1,500	Good	Golden yellow
(Stored at 40°F.)					
After 6 weeks	6.40	5.0	480	Good	Golden yellow
After 12 weeks	6.20	8.5	300	Good	Yellow
After 24 weeks	6.05	8.5	1,300	Good	Slightly discolored
After 36 weeks	6.15	9.0	1,100	Good	Slightly discolored
After 52 weeks	6.27	8.5	900	Fair	Slightly discolored
After 72 weeks	6.25	9.5	400	Fair	Slightly discolored
(Stored at 70°F.)					
After 6 weeks	6.35	5.0	200	Good	Yellow
After 12 weeks	6.08	8.75	300	Fair	Yellow
After 24 weeks	5.87	8.75	500	Fair	Definitely discolored
After 36 weeks	5.91	9.0	300	Stale	Definitely discolored (brown)
After 52 weeks	5.87	9.0	100	Stale	Definitely discolored (brown)
After 72 weeks	5.90	13.0	200	Stale	Definitely discolored (brown)
(Stored at 90°F.)					
After 6 weeks	6.26	8.0	200	Slightly bitter	Yellow
After 12 weeks	5.95	8.5	100	Stale	Dead yellow
After 24 weeks	5.80	9.5	700	Very stale	Definitely discolored
After 36 weeks	5.81	9.5	200	Very stale	Definitely discolored (brown)
After 52 weeks	5.86	8.5	500	Very stale	Definitely discolored (brown)
After 72 weeks	5.80	12.5	0	Very stale	Definitely discolored (brown)
SAMPLE B					
(Stored at —5 to —20°F.)					
At storage	6.45	7.0	4,000	Good	Golden yellow
After 6 weeks	6.35	7.0	200	Good	Golden yellow
After 12 weeks	6.20	8.0	900	Good	Golden yellow
After 24 weeks	6.20	8.0	2,240	Good	Yellow
After 36 weeks	6.39	8.0	1,850	Good	Yellow
After 52 weeks	6.55	8.0	4,000	Good	Yellow
After 72 weeks	6.50	9.0	1,200	Good	Yellow
(Stored at 40°F.)					
After 6 weeks	6.35	7.8	300	Good	Golden yellow
After 12 weeks	6.10	8.0	160	Good	Golden yellow
After 24 weeks	6.10	8.5	1,240	Fair	Yellow
After 36 weeks	6.32	8.5	1,080	Fair	Yellow
After 52 weeks	6.40	9.0	1,900	Fair	Yellow
After 72 weeks	6.32	9.0	500	Fair	Yellow

TABLE 2 *(Continued)*

Analysis of Four Samples of Powdered Egg Yolk After Periods of Storage at Different Temperatures

Storage time	pH	Insoluble material (ml.)	Total bacteria per gram	Flavor	Color
(Stored at 70°F.)					
After 6 weeks	6.30	9.5	500	Good	Golden yellow
After 12 weeks	6.20	9.5	100	Slightly stale	Slightly brown
After 24 weeks	6.15	9.5	90	Stale	Slightly brown
After 36 weeks	6.08	9.5	20	Stale	Light brown
After 52 weeks	6.00	11.0	0	Stale	Light brown
After 72 weeks	6.03	14.0	200	Stale	Light brown
(Stored at 90°F.)					
After 6 weeks	6.30	9.5	900	Good	Golden yellow
After 12 weeks	5.85	10.5	30	Slightly stale	Slightly brown
After 24 weeks	5.85	10.5	50	Stale	Brown
After 36 weeks	5.93	10.5	10	Stale, bitter	Brown
After 52 weeks	5.85	13.0	0	Stale, bitter	Brown
After 72 weeks	5.85	13.0	100	Stale, bitter	Brown
SAMPLE C					
(Stored at —5 to —20°F.)					
At storage	6.20	8.0	800	Excellent	Yellow
After 6 weeks	6.00	9.0	200	Good	Yellow
After 16 weeks	5.70	9.0	1,200	Good	Yellow
After 28 weeks	6.12	8.0	1,200	Good	Yellow
After 32 weeks	6.00	7.0	500	Good	Yellow
After 52 weeks	6.05	8.5	300	Good	Yellow
After 70 weeks	6.10	13.0	1,100	Good	Yellow
(Stored at 40°F.)					
After 6 weeks	6.02	9.0	500	Good	Yellow
After 16 weeks	5.72	9.5	1,200	Good	Yellow
After 28 weeks	6.10	8.0	900	Good	Yellow
After 32 weeks	5.95	7.5	400	Good	Yellow
After 52 weeks	6.23	10.0	500	Good	Yellow
After 70 weeks	6.05	12.0	1,100	Good	Yellow
(Stored at 70°F.)					
After 6 weeks	6.05	9.0	700	Slightly stale	Yellow
After 16 weeks	5.72	9.5	500	Stale	Slightly discolored
After 28 weeks	6.00	7.5	500	Stale	Light brown
After 32 weeks	5.82	7.5	200	Stale	Light brown
After 52 weeks	5.78	8.5	400	Stale	Light brown
After 70 weeks	5.82	14.0	400	Stale	Light brown
(Stored at 90°F.)					
After 6 weeks	6.00	10.0	1,200	Slightly stale	Slightly discolored
After 16 weeks	5.60	10.5	700	Stale	Dead yellow
After 28 weeks	5.89	7.5	100	Stale	Brown
After 32 weeks	5.71	7.5	100	Stale	Brown
After 52 weeks	5.72	8.5	200	Stale	Brown
After 70 weeks	5.78	13.5	100	Fishy	Brown
SAMPLE D					
(Stored at —5 to —20°F.)					
At storage	6.95	5.0	140	Excellent	Light yellow
After 8 weeks	6.88	8.0	4,000	Good	Light yellow
After 18 weeks	6.79	8.0	10,000	Good	Light yellow

TABLE 2 *(Concluded)*

Analysis of Four Samples of Powdered Egg Yolk After Periods of Storage at Different Temperatures

Storage time	pH	Insoluble material (ml.)	Total bacteria per gram	Flavor	Color
After 24 weeks	6.80	9.0	.....	Good	Light yellow
After 40 weeks	6.90	9.25	38,000	Fair	Light yellow
After 72 weeks	6.90	8.5	45,000	Fair	Light yellow
(Stored at 40°F.)					
After 8 weeks	6.90	9.0	7,000	Good	Light yellow
After 18 weeks	6.90	9.0	10,000	Good	Light yellow
After 24 weeks	6.85	10.0	.....	Fair	Light yellow
After 40 weeks	7.00	9.25	21,600	Fair	Light yellow
After 72 weeks	6.90	7.0	26,000	Fair	Light yellow
(Stored at 70°F.)					
After 8 weeks	6.90	9.0	15,000	Slightly stale	Light yellow
After 18 weeks	6.85	10.5	7,500	Stale	Slightly discolored
After 24 weeks	6.75	10.5	.....	Stale	Slightly discolored
After 40 weeks	6.50	16.0	5,200	Stale	Slightly discolored
After 72 weeks	6.50	13.0	7,000	Stale	Slightly discolored
(Stored at 90°F.)					
After 8 weeks	6.88	9.2	32,500	Slightly stale	Slightly discolored
After 18 weeks	6.70	11.0	5,000	Stale	Strongly discolored
After 24 weeks	6.57	12.0	.....	Stale, bitter	Strongly discolored
After 40 weeks	6.25	13.0	600	Stale, bitter	Strongly discolored
After 72 weeks	6.28	16.0	700	Stale, bitter	Strongly discolored

ing from a deep yellow or orange to a dead yellow shade. The flavor of the product also became less desirable. Changes occurring at 40°F. or lower were slight. The sample containing the skim-milk solids deteriorated more rapidly during storage than any of the all-egg-yolk samples.

It was interesting to note that in the case of all samples the bacteria present were somewhat inactivated by storage at room temperature or above. In general, the counts were higher on the samples stored at 40°F. or lower.

COMPARISON OF POWDERED EGG YOLK IN ICE CREAM

No attempt will be made to present the data secured in connection with the use of powdered egg yolk in ice cream. Storage at any of the temperatures studied did not prevent the egg yolk from improving mix whipping. The storage of the egg yolk did not change the way in which it affected the body of the ice cream or its melt-down characteristics. Though discriminating judges were able to detect a slight difference in the flavor of ice cream when using egg yolk stored for three months at 90°F., most consumers were unable to detect any difference. This was true even after the egg yolk had been stored for 18 months. The off-flavor that developed in the egg yolk stored at room temperature or above, while very evident when the yolk was judged undiluted, was not a serious factor when the yolk was added to the mix at the rate of 0.5 per cent. For best results, however, it would be better to store the dried egg yolk at 40°F. or lower, unless it is to be used

within three months. A low storage temperature is more important in the case of an egg-yolk and milk-powder combination because of the more rapid development of a stale storage flavor in a mixture of this type.

SUMMARY

The data obtained may be summarized as follows:

At room temperature or above the pH of powdered egg yolk decreased as the time of storage of the powder increased. Little change took place at 40°F. or lower.

The solubility of egg yolk decreased in storage particularly at the higher storage temperatures.

The total bacteria count tended to decrease as the temperature and time of storage increased. Samples of egg powder stored at —5°F. (below zero) had a higher plate count per gram than samples stored at 90°F., even after a storage period of one and one-half years.

In samples stored at room temperature or above a distinct color change was evident at the end of six weeks, becoming more pronounced as the storage time increased. There was a direct relationship between the temperature of storage and the color of the powdered egg. Dried eggs stored at 40°F. or below retained their color better than eggs stored at higher temperatures.

Powdered egg yolk stored at or above 70°F. developed a stale flavor after six to 12 weeks of storage. The degree of undesirable flavor developed was proportional to the time and temperature of storage of the powder.

Powdered egg yolk deteriorated rapidly when milk solids were present.

While the best-flavored ice cream resulted when using powdered egg yolk stored at 40°F. or lower, that stored at temperatures as high as 90°F. for 18 months was used to the extent of 0.5 per cent in mix without serious effect upon the flavor of ice cream.

The time and temperature of storage were not found to be related to the effect that egg yolk may have upon the whipping properties of the mix or the melt-down characteristics of the ice cream.

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VITAMIN CONTENT OF PRUNES AS AFFECTED BY STORAGE AND OTHER FACTORS[1]

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A new study of the nutritive properties of prunes was undertaken in this laboratory recently, to determine more definitely the place of this cheap and abundant fruit crop in the diet and to discover effects of methods of storage and handling on these properties. The study was divided into three parts: (1) the assay of freshly harvested and variously stored prunes for vitamin content; (2) the determination of the anti-anemia value of prunes and prune products; and (3) the evaluation of prunes and prune paste as a constituent of dog foods. The results of each of these studies are reported separately, and this paper covers only the first.

Prunes from the crops of 1938, 1939, 1941, and 1942 were used, nearly all of the French type and all grown in northern California.

The 1938 prune samples were commercial fruit, all produced in the Santa Clara valley by one grower.[3] They were French prunes, dehydrated at 73.9°C.(165°F.), humidity 105-110, for 24 hours, were not lye-dipped, and had been stored in bins for 10 months. Two sizes were used, 50-60 and 120 to the pound, in order to detect any changes in vitamin concentration owing to size of fruit.

The 1939 samples, all of size 50-60 per pound, were taken from a large mixed shipment,[4] also largely grown in the Santa Clara valley, were mostly but not all dehydrated, and were used for the experiment in the use of prune-pit oil as a protective spray for the fruit during storage as well as for vitamin assay and other purposes.

The 1941 prunes came from the Napa and Santa Clara valleys, and the 1942 fruit from Walnut Creek.

PREPARATION AND USE OF PIT OIL

The pits were removed from dried prunes by hand in the laboratory, crushed in a grinder, and extracted twice with 10 times their volume of ethyl ether. Practically all of the oil extracted was removed by the first lot of ether. This proved to be a light reddish oil of pleasant odor and

[1] This study was made possible by a grant from Prune Proration Zone No. 1 to the University of California.

[2] Assistance in this study was rendered by Work Projects Administration Official Project 65-1-0862, Unit A-24, assigned to the University of California.

[3] Our thanks are extended to N. C. Leone, California State Department of Health, for securing these samples, and to the grower, Douglas Sim, San Jose, California.

[4] We acknowledge with gratitude the assistance of Libby, McNeil and Libby of San Francisco and Oakland in procuring, treating, and storing the prunes used in this experiment.

taste. The yield was about seven per cent of the crushed pits and kernels. The color was chiefly due to the carotenoids of the adhering prune flesh and was therefore variable, but the kernel oil itself carried little carotene. The oil had the equivalent of 43 U.S.P. units of vitamin A per gram (Table 2). A later extraction of commercially produced and crushed pits, by-product of a pitted-prune plant, provided a less desirable oil, darker in color and less agreeable in odor and taste. This was the product used in the storage and vitamin assay experiment. The seven-per cent yield of pit oil obtained in the first experiment represented a nearly quantitative extraction since the kernels, which were 18 per cent of the crushed pits, contained about 40 per cent oil, Giaja (1927).

The pretreatment and storage experiment was planned to determine the effect of pit-oil spraying and of processing of prunes upon the keeping

TABLE 1

Plan of Storage Study

Treatment of prunes	Lot	Storage	Quality	Water content
				pct.
Oil sprayed, processed	A	None	Excellent	33.4
	B	7 mo. in bin	Good	17.5
	C	7 mo. in box	Good	21.0
Not oil sprayed, processed	D	None	Excellent	30.4
	E	7 mo. in bin	Fair	17.0
	F	7 mo. in box	Good	21.0
Oil sprayed, not processed	G	None	Excellent	29.2
	H	7 mo. in bin	Excellent	18.0
	I	7 mo. in box	Excellent	18.8
Not oil sprayed, not processed	J	None	Excellent	26.4
	K	7 mo. in bin	Poor	18.0
	L	7 mo. in box	Poor	19.5

quality, particularly as to sugaring, of fruit stored in bins or sacks and in boxes under ordinary warehouse conditions. A large lot of prunes was divided so as to obtain representative samples; part were sprayed with a very thin coating of the pit oil and stored in sacks and tightly closed wooden boxes holding 25 pounds and part were removed for immediate assay. Also some of each lot was processed, that is, steamed before spraying and storing; the scheme is shown (Table 1). Water, sugar, carotene, thiamin, riboflavin, pyridoxine, and "filtrate-fraction" (pantothenic acid, chiefly) activity determinations were made on most of these lots of prunes, cooked and uncooked, immediately after their preparation in November, 1939, and again in June, 1940. In February, 1941, some of the samples were again examined for appearance and flavor.

Sugaring and shrivelling were most pronounced in the fruit stored in sacks rather than boxes and in the lots which were not oil sprayed and not processed (Figs. 1 and 2). Moreover, some of the samples which were removed from the warehouse for use in vitamin assay and were kept in the laboratory in glass jars for several months, were found to remain free of insect infestation only in case the prunes had been oil treated. All

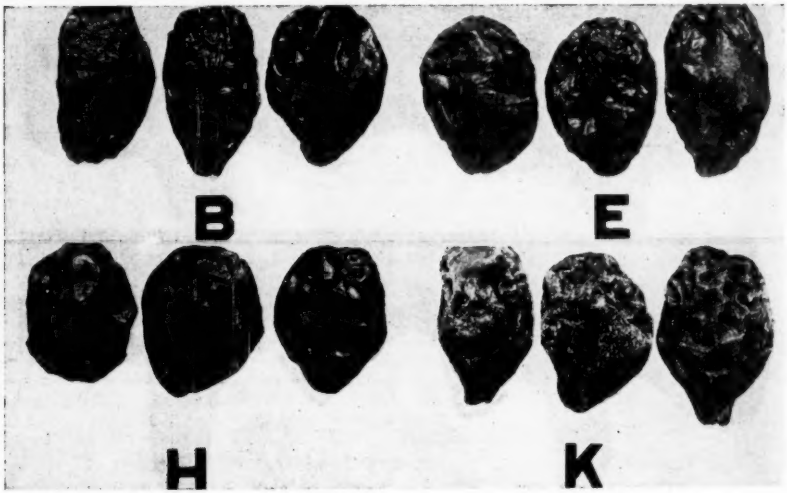

FIG. 1. Effect of pretreatment on prunes stored in bins or sacks for seven months.

1939 CROP

Lot B—Processed and sprayed with prune-pit oil
Lot H—*Not* processed but sprayed with prune-pit oil
Lot E—Processed, *not* sprayed with prune-pit oil
Lot K—*Not* processed, *not* sprayed with prune-pit oil.

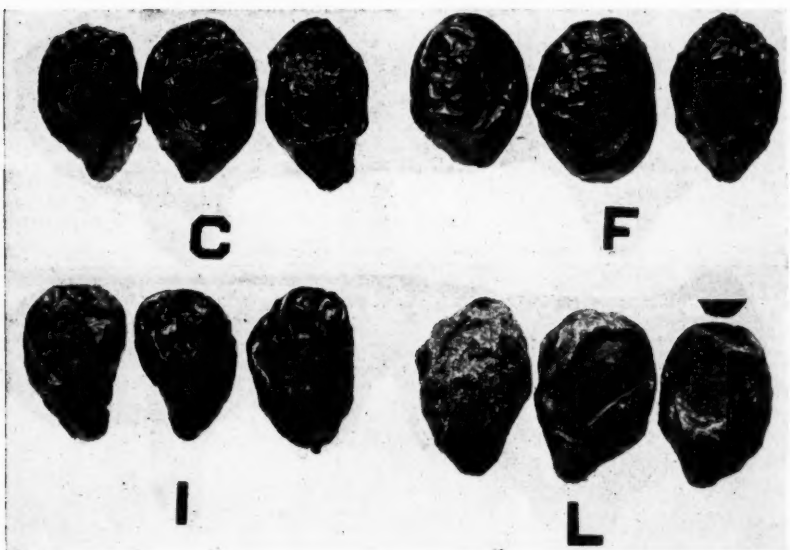

FIG. 2. Effect of pretreatment on prunes stored in tightly closed boxes for seven months.

1939 CROP

Lot C—Processed and sprayed with prune-pit oil
Lot I—*Not* processed but sprayed with prune-pit oil
Lot F—Processed, *not* sprayed with prune-pit oil
Lot L—*Not* processed, *not* sprayed with prune-pit oil.

the samples which were stored in burlap sacks, whether oil sprayed or not, were infested, but the oil-treated samples were less severely infested. Only Lots C and I, which had been oil treated and stored in tight boxes, were free from infestation. These as well as the other oil-sprayed lots also remained relatively free from surface sugaring.

The moisture content of all samples, as might be expected, was reduced during storage to 17 to 19 per cent, but the oil-treated fruit retained at first about four per cent more water than the untreated. The prevention of surface sugaring by the oil in spite of this uniform dehydration in storage would indicate that sugaring is not chiefly a drying action. The incrustation appeared to be made up of sugar crystals and yeasts.

This practical use of pit-oil coating of prunes was first suggested some years ago by R. S. Hiltner and E. M. Mrak, Mrak and Long (1941). Only oil derived from the fruit to be treated can be used under present federal food laws. Pit oil is exceptionally well adapted for this purpose but care must be taken to use clean, fresh oil which will not rancidify in storage. No further rancidity developed in the study here reported, but the oil was not wholly free from rancidity when it was applied.

VITAMIN ASSAYS

Some years ago certain of the vitamin values of California prunes were ascertained by biological means in this laboratory. Ascorbic acid was found in the fresh frozen fruit (79.5 per cent water), in amounts which may be estimated as 50 to 85 mgm. per 100 gm., and in the sulfured, lye-dipped, dehydrated form (19 per cent water) of the same fruit, 160 to 200 mgm. per 100 gm., indicating nearly complete retention of the vitamin under these conditions—Morgan and Field (1929), and Morgan, Field, and Nichols (1931). The sun-dried, sulfured, lye-dipped prunes retained some but not all of the vitamin. All unsulfured products, both dehydrated and sun-dried, were without antiscorbutic activity. This was the first recognition of the protective effect of sulfur dioxide against the oxidation of ascorbic acid. Since commercially produced dried prunes are seldom sulfured or dehydrated, they cannot be considered as having antiscorbutic value. We have not therefore continued work on this vitamin.

VITAMIN A

The vitamin A activity of fresh and dried prunes, determined by the rat-growth method, Morgan and Field (1930), was also found to be retained more effectively by the sulfured, dehydrated fruit than the unsulfured product. This protection of the carotene against oxidation by sulfur dioxide is not unexpected. Storage did not diminish the vitamin A activity of the dried prunes.

The vitamin A value of the carotene present in prunes was determined by the U.S.P. XI biological assay method (Table 2). It was interesting to note the greater value of the small-sized fruit, 34 as against 24 units of vitamin A per gram of prune solids. An attempt was made to determine whether this signified a greater concentration of carotene in the skin by feeding skin and flesh separately; no difference was found, however. The

dry prune pulp left after thorough extraction of the water-soluble constituents appeared to retain all the vitamin A value of the original fruit. This was especially surprising since some slight fermentation had taken place previous to drying. It indicates, moreover, that so-called prune juice made by water extraction of the dried fruit is not likely to carry any vitamin A value, a fact previously demonstrated in this laboratory.

Some question arises as to the significance of chemical assay of fruits for beta-carotene. Mackinney, Aronoff, and Bornstein (1942) have pointed out certain discrepancies between provitamin A carotenoids and biologically effective vitamin A values of plant materials. Prunes, both fresh and processed, yield their pigment readily but the air-dried type, 18 per cent

TABLE 2

Vitamin A Value of Prunes

Crop year	Product	Total fed in 28 days, dry solids	Gain in weight in 28 days	U.S.P. units vitamin A per gm. solids
		gm.	*gm.*	
1938, stored 10 months	Whole prune flesh, size 30-40	2.1	23	24
	Skins only	2.0	20	23
	Flesh, no skins	3.4	38	25
	Whole prune flesh, size 120	2.3	35	34
	Reference cod-liver oil I	19 (mgm.)	25	3,000
	Prune pulp, after water extraction	2.5	40	36
	Prune-pit oil, ether extracted	1.3	26	43
1939, stored 10 months	Prune paste, no pits	2.4	29	23
	Whole prune paste, including pits	2.4	43	32
1939, stored 8 months	Prune flesh, 50-60 size (Lots B, H, K)	2.3	29	24
	Reference cod-liver oil I	19 (mgm.)	30	3,000

moisture or less, gives up no carotenoids to petroleum ether. The addition of hot water produces their immediate release. Fresh Imperial prunes contained 0.0041 and 0.0037 per cent beta-carotene on a moisture-free basis, and market samples of processed prunes, 0.001 to 0.003 per cent. By definition the International unit of vitamin A has the biological value of 0.6 microgram of beta-carotene. The commercial samples would therefore contain 16 to 50 units per gram. But even vitamin A-depleted rats do not respond to beta-carotene and animal vitamin A in the ratio as set forth for the International standard. However, the range of values quoted for prunes by Mackinney, Aronoff, and Bornstein (1942) from petroleum ether extraction and elution from a magnesium carbonate column, 16 to 50 units per gram, covers adequately the range obtained by our rat-assay trials, 23 to 34 units per gram.

THIAMIN

The vitamin B_1 (thiamin) content of dried-prune flesh, 50-60 size fruit, 1933 crop, was found by Morgan, Hunt, and Squier (1935) to be about 0.7 I. U. or 2.0 micrograms per gram as fed, or 0.2 milligram per 100 grams of market prunes. This value was later confirmed by Booher and Hartzler (1939), using random market samples in Washington, D. C.

Four of the stored samples described (Table 1) were assayed by the rat-growth method, using a basal diet containing 15 per cent autoclaved yeast as source of the B vitamins other than thiamin and crystalline thiamin chloride fed to the control groups. As shown (Table 3) all of these samples, even after 10 months' storage, contained more than five micrograms of thiamin per gram of solids, or 0.3 to 0.4 milligram per 100 grams of market dried prunes having 20 to 30 per cent moisture.

TABLE 3

Thiamin Content of Prunes Determined by Rat-Growth Method

Lot	Description	Amount fed daily for 28 days	Total gain in weight	Thiamin (micrograms per gram)	
				As fed	Solids
		gm.	*gm.*		
B	1939 crop, dehydrated, stored 8 mo. in sacks, oil sprayed, processed	.75	43	4.5	
				5.4	5.1
		.50	16	3.5	
E	Same, not oil sprayed	.75	38	5.1	
				4.7	5.2
		.50	25	4.9	
H	Same, not processed	.75	43	5.4	
		.50	17	3.2	5.2
K	Same, not processed, not oil sprayed	.75	34	4.2	
		.50	29	5.4	5.8
		micrograms			
	Thiamin chloride	2	20	....	....
		3	32	....	....
		6	60	....	....

The prunes of both the 1938 and 1939 crops therefore appeared to contain about 60 per cent more thiamin than did those of the 1933 crop. The prunes here reported upon, however, were all dehydrated and carefully stored samples, whereas the 1933 samples were of random origin and were probably mostly sun-dried, the procedure in general use at that time.

RIBOFLAVIN

The vitamin G (riboflavin) content of prunes of the 1933 crop, Morgan, Hunt, and Squier (1935), was found to be "266 Sherman units per 100 gm."

Since the "Sherman unit" of vitamin G is now thought to represent two to four micrograms of riboflavin, the 1933 prunes contained five to 10 micrograms of riboflavin per gram. In the same study wheat germ, dried whole eggs, and dried whole milk yielded only slightly larger riboflavin values than the prune flesh; dried spinach had about twice the amount; dried beef liver and brewery yeast had about four times as much. These latter assays have been confirmed in this and other laboratories by comparison with the growth of rats fed pure riboflavin.

New determinations of riboflavin in dried prunes of the 1938, 1939, and 1942 crops were undertaken by the biological method, using young rats. Five assays in all were made. The basal diet was that in use in this

laboratory for some years. It contains washed casein, 22;[5] salt mixture, Hubbell *et al.* (1937), 2.5; hydrogenated cottonseed oil, 9; sucrose, 66.5. Vitamins A and D were given separately in carotene-reinforced, cod-liver oil, four drops daily. The B vitamins other than riboflavin were supplied separately in solution in the daily amounts per rat: thiamin chloride, 10 micrograms; pyridoxine[6] 15 micrograms; filtrate-fraction concentrate[7] from yeast equivalent to 80 to 100 micrograms pantothenic acid, the latter solution providing the unknowns as well.

In thiamin, pyridoxine, and pantothenic acid and filtrate-factor assays the same procedure was used except that the vitamin being measured was omitted and 20 micrograms of riboflavin were added. In some assays crystalline calcium pantothenate, 80 to 100 micrograms, was given instead of the yeast-filtrate concentrate.

BIOLOGICAL ASSAY FOR RIBOFLAVIN

Standard weanling rats were kept on the basal diet plus vitamin supplements until the weight curve "plateaued" in the case of riboflavin usually after four to five weeks. One-half to one and one-half grams of prune flesh was then given daily and the resumption of growth compared with that of carefully selected litter-mate groups receiving known amounts of the crystalline vitamin. An equal number of animals was left without source of the missing vitamin. The groups contained five to nine animals.

In some cases the prune doses, after being weighed, were cooked, just previous to feeding, for about 30 minutes in a small amount of water which was fed with the prunes.

The usual signs of riboflavin, pyridoxine, or filtrate-factor deficiency were exhibited by the respective negative groups, and normal or graduated growth and normal appearance were shown by those which received all supplements.

There was some variation owing to crop year or to method of drying in the riboflavin value of the prunes (Table 4). The 1938 crop, although examined 10 months after harvest, carried as much riboflavin as did the 1939 crop and nearly twice the probable value of the 1933 and 1942 crops. None of the other vitamins assayed had so large a range owing to crop year. Some factor of rainfall, sunshine, or other unknown climatic influence may affect the production or retention of riboflavin of prunes. The fact that the 1933 prunes were sun-dried, the 1938 all dehydrated, and

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[5] Most of the washed casein used in this study was supplied through the courtesy of P. D. V. Manning and the Western Condensing Company, San Francisco.

[6] Merck & Co., Rahway, N. J., generously provided the pyridoxine and calcium pantothenate used in this study.

[7] The method of preparation of this concentrate was as follows: Brewer's yeast was alternately extracted with 100 per cent and 60 per cent acetone four times, using each time five volumes of the solvent. The extract was then reduced under vacuum until the volume in milliliters was equal to the weight of the yeast in grams. This concentrate was then shaken with fuller's earth, 100 gm. per liter, filtered and the shaking repeated three times with 300 gm. fresh portions of fuller's earth per liter. The filtrate was finally concentrated until each milliliter represented eight to 16 grams of the original yeast. Repeated microbiological assays of these preparations have indicated that they contain 0.5 to 0.8 mgm. of pantothenic acid per gram.

the 1939 nearly all dehydrated may be one reason for difference. Certainly riboflavin might well be expected to be adversely affected by long exposure to sunlight.

There was no consistent loss owing to cooking, to storage, or to treatment previous to storage; although one lot, A-B, processed before storage, appeared to lose about a third of its riboflavin activity in storage.

MICROBIOLOGICAL DETERMINATIONS [8] OF RIBOFLAVIN AND OTHER VITAMINS

Late in 1941 when the use of *Lactobacillus casei* as test species for the determination of riboflavin had become fairly well standardized, a new assay of 1941 prunes was undertaken. The prunes, French, Imperial, and

TABLE 4

Riboflavin, Pyridoxine, and Pantothenic Acid (and "Filtrate-Factor") Contents of California Prunes [1]

Lot	Description	Size [2]	Moisture	Riboflavin	Pyridoxine	Pantothenic acid [3]
			pct.			
T	1938 crop, stored 10 months.....	30-40	18.8	1.6	1.3	9.7
P		120	16.8	1.2	1.3	6.8
J	1939 crop, freshly harvested.....	50-60	25.4	1.2	1.0	7.4
	Same, cooked.....	50-60	25.4	1.3	0.9	7.0
K	Same, stored 8 months.....	50-60	18.0	1.4	1.0	7.0
G	1939 crop, freshly harvested, oil sprayed.....	50-60	29.2	1.2	1.0	5.5
	Same, cooked.....	50-60	.....	1.4	0.5	3.7
H	Same, stored 8 months.....	50-60	18.0	1.2	1.1	6.0
A	1939 crop, freshly harvested, oil sprayed, harvested.....	50-60	33.4	1.8	1.5	6.0
	Same, cooked.....	50-60	.....	1.2	1.0	7.0
B	Same, stored 8 months.....	50-60	17.5	1.3	1.5	5.6
D	1939 crop, freshly harvested, processed.....	50-60	30.4	1.3	0.6	9.7
	Same, cooked.....	50-60	.....	1.0	0.6	9.4

[1] Given in milligrams per 100 grams of solids. [2] Number of prunes per pound. [3] All "filtrate-fraction" value of the prunes is expressed as pantothenic acid.

Robes, were obtained from growers in the Napa and Santa Clara valleys and were examined fresh and after commercial tunnel dehydration. The method used was that of Snell and Strong (1939), and numerous procedures for extraction of the vitamin from the fruit were tried. Grinding in a mortar, in a food chopper, or in an electric blender and shaking with distilled water, hot or cold, by hand or in a rotary shaker for three hours or with acid-alcohol in the shaker for three hours, were tried on the fresh prunes. Shaking in the rotary shaker for three hours in brown bottles with distilled water at 15.6°C.(60°F.) produced the largest amount of extraction. All the values in these assays (Table 5) are extremely low as compared with the results of the biological assays previously obtained.

[8] We acknowledge valuable assistance rendered by Marian B. Smith, Marian C. Hunner, and Relda Cailleau in the microbiological studies.

Further work was then done only on the dehydrated French prunes from the Napa valley. Again numerous methods of grinding and extracting were used. It was found that steaming the fruit for one hour nearly doubled the amount of riboflavin extracted as did the use of 0.04 N H_2SO_4 instead of distilled water. The total riboflavin again was found to be much lower than in the biological assays although equivalent to the quantity present in the corresponding fresh fruit, 0.16 mgm. per 100 gm. solids.

TABLE 5

Riboflavin Content of Fresh and Dehydrated Prunes, 1941 Crop, by Microbiological Method[1]

Prunes	Range	Mean	
		Sample	Solids
	mgm./100gm.	*mgm./ 100 gm.*	*mgm./ 100 gm.*
French, fresh			
Napa Valley	.022-.052	.038	.15
Santa Clara	.023-.053	.039	.16
Imperial, fresh			
Napa Valley	.018-.035	.022	.09
Santa Clara	.020-.043	.032	.13
Robes, fresh			
Napa Valley	.007-.029	.016	.06
French, dehydrated			
Napa Valley	.100-.145	.120	.16

[1] There were six to 12 determinations made on each sample, but no enzymatic treatment for extraction was used.

When the 1942 crop was available French prunes from Walnut Creek were obtained fresh and portions were dehydrated[9] after lye-dipping in a tunnel dehydrator (drying time 20 hours) at an average drying ratio of 3.2:1. Another portion was sun-dried after lye-dipping (drying time nine days) at an average drying ratio of 3.0:1.

Samples of each of these three lots were prepared by mixing in the mixer-blender with water and aliquots treated with various extractants. Riboflavin, pantothenic acid, and nicotinic acid were determined microbiologically on these extracts. Digestion of the prune purée with 1.2 per cent pancreatin considerably increased the yield of riboflavin (Table 6), possibly because of its release from the protein-bound form. Nevertheless the largest of these values, 0.29 mgm. per 100 gm. of solids, is only one-third to one-tenth the amount found by biological assay of earlier crops (Table 4). An even greater discrepancy as to pantothenic acid appeared, 0.4 mgm. instead of five to nine mgm. per 100 gm. solids. The microbiological method used for the latter determination was that of Strong, Feeney, and Earle (1941).

Two experiments were next tried in the hope of reconciling these differences. Riboflavin and pantothenic acid assays by the improved biological method were made on the same 1942 prunes and new microbio-

[9] We acknowledge the assistance of Hazel Friar and Edward G. Balog of the Fruit Products Division, College of Agriculture, University of California, in the preparation of the 1942 dehydrated and sun-dried samples.

TABLE 6

Microbiological Assay for Riboflavin, Pantothenic Acid, and Nicotinic Acid of Fresh, Sun-Dried, and Dehydrated 1942 Crop French Prunes[1]

Prunes	Extraction medium	Treatment	Riboflavin		Nicotinic acid		Pantothenic acid	
			Sample	Solids	Sample	Solids	Sample	Solids
Fresh, 74.7 per cent moisture	.04 NH_2SO_4	Steamed 1 hr.	.023	.091	0.55	2.18	....	....
	Phosphate buffer, pH 8.2	Steamed 1 hr.	.023	.091	0.55	2.18	....	....
	Phosphate buffer, pH 7.8	Steamed 1 hr.	.023	.091	0.55	2.18	....	....
	Acetate buffer, pH 4.5	Steamed 1 hr.	.025	.098	0.55	2.18	....	....
	Phosphate buffer, pH 8.2	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	.073	.288	0.60	2.37	....	....
	Acetate buffer, pH 4.5	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	.054	.213	0.60	2.37	....	....
Derydrated, 17 per cent moisture	.04 NH_2SO_4	Steamed 1 hr.	.085	.101	1.80	2.17	....	....
	Phosphate buffer, pH 8.2	Steamed 1 hr.	.080	.096	1.90	2.27	....	....
	Phosphate buffer, pH 7.8	Steamed 1 hr.	.090	.108	1.80	2.17	....	....
	Acetate buffer, pH 4.5	Steamed 1 hr.	.070	.084	1.80	2.17	.33	.40
	Phosphate buffer, pH 8.2	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	.220	.265	1.80	2.17	....	....
	Acetate buffer, pH 4.5	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	1.700	.204	1.80	2.17	.33	.40
Sun-dried, 18.4 per cent moisture	Acetate buffer, pH 4.5	Steamed 1 hr.	.050	.061	.....	.....	.33	.40
	Phosphate buffer, pH 8.2	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	.190	.230	.....	.....	....	....
	Acetate buffer, pH 4.5	Autolyzed 2 hr. with 1.2% pancreatin, steamed 1 hr.	.140	.170	.....	.....	.33	.40

[1] Given in milligrams per 100 grams.

logical tests of the prunes were made with accompanying recovery of added riboflavin.

Choline, five mgm. daily, and crystalline calcium pantothenate were used in the new biological assays, and several levels of pure riboflavin or pantothenate were fed the control groups so that a curve of response was charted making it possible to read directly the riboflavin or pantothenic acid content of the prune doses fed; the results of these tests are shown (Table 7). Thus 0.7 to 0.8 mgm. of riboflavin per 100 gm. of solids were

TABLE 7

Riboflavin and Pantothenic Acid Contents[1] of 1942 Prunes Obtained by Rat-Feeding Methods

Prunes	Riboflavin		Pantothenic acid	
	Sample	Solids	Sample	Solids
Dehydrated	.59	.71	....	....
Sun-dried	.65	.79	2.6	3.2

[1] Given in milligrams per 100 grams.

found by rat feeding in the same prunes which by microbiological means yielded only 0.2 mgm. per 100 gm. of solids. Likewise the comparison as to pantothenic acid is seen to be 3.2 by biological and 0.4 by microbiological methods. The 1942 prunes were lower in riboflavin by 30 to 50 per cent than those of 1938 and 1939 but of about the same value as the 1933 samples. The pantothenic acid content of the 1942 fruit cannot be adequately compared with those of the earlier crops because all "filtrate-factor" value of the prunes was expressed as pantothenic acid in the earlier assays but only pantothenic acid itself is operative in the 1942 value, which is therefore smaller.

Riboflavin recovery by microbiological method was attempted by preparation of a large amount of sample which was divided into aliquots, extracted in various ways, and riboflavin added to the extracts either after extraction directly to the assay tubes or to the samples just before extraction. The recovery of added riboflavin was found to vary from —2 to 66 per cent of that found when the prune extract was present and from 57 to 95 per cent when it was not (Table 8). Likewise the digestion with pancreatin, particularly in the presence of prune extract, decreased the recovery somewhat. Nevertheless the yield of prune riboflavin alone was better (Table 6) after pancreatin autolysis. Possibly the destruction produced by the pancreatic enzyme was more than counterbalanced by the greater release of bound riboflavin which it accomplished. This further complicates the interpretation of the losses shown (Table 8).

Similar discrepancies between microbiological and biological assay for riboflavin have been noted in this laboratory in other foods. For example, fresh and dehydrated beef samples were found to yield only one-half the riboflavin value by microbiological method compared with the biological method; and fresh and dehydrated green peas, from one-half to one-fifth. The difficulty of extracting all riboflavin or other vitamins from the food by the usual methods advised probably accounts for most of the difficulty

although there may be in some foods, as in prunes, constituents which destroy the value of riboflavin (or pantothenic acid) for microorganisms under the conditions of the assay but not for use by animals, such as the rat.

In order to assess the probable riboflavin content of the 1941 prunes comparison may be made with the 1942 dehydrated samples, assayed by the same methods used with the 1941 fruit. The 1942 fresh prunes, with simple acid extraction, yielded 0.09 mgm. per 100 gm. solids; the

TABLE 8

Recovery of Riboflavin From Prune Extracts

Extract	Riboflavin added		Enzyme preparation	Recovery of added riboflavin in extraction media		
	Amount (micrograms per ml.)	Time		.04 N H_2SO_4	Phosphate buffer, pH 8.0	Acetate buffer, pH 4.5
				pct.	*pct.*	*pct.*
Prunes, 10 gm. per 100 c.c. extractant	0.1	Assay tube	None	40 [1]	66	46
	0.1	Extraction	None	39	49	46
	0.1	Assay tube	1.2% pancreatin	35	33	54
	0.1	Extraction	1.2% pancreatin	31	—2	49
No prunes	0.1	Extraction	None	89	88	95
	0.1	Assay tube	1.2% pancreatin	85	57	68
	0.1	Extraction	1.2% pancreatin	78	72	72

[1] Each figure represents the average of six experiments using three dilutions of the extract.

dehydrated and sun-dried, 0.1 and 0.06, indicating little loss at least in dehydration. But the 1941 fruit contained 0.16 mgm. of riboflavin per 100 gm. solids, both fresh and dehydrated. Probably, therefore, the 1941 prunes contained about 50 per cent more riboflavin than the 1942 samples, that is, 1.0 to 1.1 mgm. per 100 gm. solids or 0.7 to 0.8 per 100 gm. market fruit.

Mackinney and Sugihara (1942), using the fluorimetric method of Conner and Straub (1941) and pectinol in addition to clarase for extraction on 1941 dried prunes, obtained the maximum value of 0.16 mgm. per 100 gm. quite like that shown (Table 5) and obtained by the unmodified microbiological assays.[10]

Nicotinic Acid: The nicotinic acid of the prunes, determined by the microbiological method of Snell and Wright (1941), appeared to be fairly constant, not affected by method of extraction or by dehydration. Since

[10] New fluorimetric determinations made on 1943 samples in this laboratory by Lowell E. Kidder by the method of Peterson, Brady, and Shaw (1943) and checked by lumichrome oxidation technique after extraction of the prunes with 0.04 NH_2SO_4, autoclaving at 15 pounds for 20 minutes and either with or without pancreatin digestion, yielded riboflavin values similar to those obtained by rat feeding, 0.55 to 0.59 mg. per 100 gm. solids. Microbiological assay of the same samples indicated only 0.08 mg. per 100 gm. solids.

no biological or chemical examination for this vitamin was attempted, there is no certainty that this value is the true one.

Pyridoxine: By suitable manipulation the biological method used for riboflavin assay yielded satisfactory values for the pyridoxine content of the prunes. This varied from six to 15 micrograms per gram of solids. This range may be compared with that obtained by Henderson, Waisman, and Elvehjem (1941) by a similar method for beef muscle, 12 to 16; pork, 7 to 28; and wheat germ, 15.9.

The control groups, which were given the same basal ration, were taken from the same litters as the rats fed the prunes and were given 8, 10, 20, or 30 micrograms of crystalline pyridoxine hydrochloride or an eluate standardized against the pyridoxine.[11] Maximum growth resulted at the level of 12 to 15 micrograms daily. The groups deprived of pyridoxine exhibited the specific skin symptoms characteristic of this deficiency, and depletion was not assumed until growth ceased and such symptoms began to appear. This required from four to six weeks and varied with each assay. A small and variable loss of pyridoxine appeared to occur in the cooked prunes but none occurred in storage.

Pantothenic Acid and Other Filtrate Factors: The same basal diet and supplements used in the riboflavin and pyridoxine experiments were used except that the yeast-filtrate concentrate was omitted and the riboflavin and pyridoxine given. In some cases crystalline calcium pantothenate was given the control groups in addition to, or instead of, the filtrate concentrate. During the six to eight weeks of feeding following four to six weeks of depletion the response of these groups appeared to be largely due to the pantothenic acid. Maintenance for a longer period resulted in slowing of growth of the animals given the pantothenic acid only but not in those given the filtrate concentrate. Greying of the fur of the deficient animals was usually observed and this was remedied slowly and incompletely by the prune doses as well as by the pantothenic acid. The yeast-filtrate concentrate, on the other hand, usually produced rapid and permanent darkening of the fur. Possibly one or another of the unknowns apparently needed for full utilization of pantothenic acid may be lacking in the prunes, Martin (1942).

A group of five rats, for example, was kept on the filtrate fraction-deficient diet for about four months until their fur was uniformly and completely greyed. Prune meat, 1.5 to three grams daily, was then offered to three of the rats with such prompt response that within 40 days they had gained 81 grams and their fur was largely re-pigmented. The other two failed, continued grey, and died of the deficiency.

The range of pantothenic acid and other filtrate-factor values for the prunes is large, 32 to 97 micrograms per gram solids. None of the conditions examined had a consistent effect upon these values. Crop year

[11] In some of the prune assays an eluate from adsorbates from autolyzed wheat germ or yeast was used as the source of pyridoxine. These eluates were assayed biologically for riboflavin and "filtrate fraction" as well as for "vitamin B_6." Their value as the source of pyridoxine was determined in these assays by comparison with crystalline pyridoxine.

(except for 1942), length of storage, cooking, or size of fruit could not be said to dominate the "filtrate-factor" activity of the fruit. It is natural that the range of response was large since the single substance, pantothenic acid, was not the only thing measured, again except for the 1942 samples. There is little doubt, however, that prunes have a rather large pantothenic acid content, of the magnitude of three to nine mgm. per 100 gm. solids.

The range of pantothenic acid activity found is quite different from that reported by Jukes (1941) for cull prunes using the chick assay—less than 0.6 microgram per gram on the undried basis. There were, of course, large differences in quality and origin of the prunes used in the chick and rat experiments here compared. Moreover, the full "filtrate-fraction" activity of the prunes is expressed as pantothenic acid (Table 4), which would tend to raise the latter value.

The nicotinic acid value, approximately 0.5 mgm. per 100 gm. dry prunes, corresponds very well with that found by Russell, Taylor, and Beuk (1943) in canned prune and tomato juices, but is larger than figures quoted for other fruits except bananas and avocados.

PRUNE JUICE

An effort was made to find out how much of the water-soluble vitamins are extracted when dry prune pulp is treated with warm water, three volumes, filtered through cloth, and the filtrate concentrated so that each milliliter is equivalent to one gram of the original prune pulp. One-third to one-half of the riboflavin, nearly all of the pyridoxine, but very little of the pantothenic acid was extracted. When no pulp is present in the "prune juice" none of the vitamin A is present, since the latter is not water-soluble.

VITAMIN E IN PRUNE PULP AND PIT OIL

A few experiments were made to determine the vitamin E value of prune pulp and the pit oil. The diet containing casein, 31; cornstarch, 33; yeast, 10; irradiated lard, 22; salts, 4, was supplemented with cod-liver oil reinforced with carotene to provide 150 to 300 I. U. of vitamin A and 10 of vitamin D per rat per day. The female rats were placed on this diet at weaning, vaginal smears were examined daily, and the rats were mated with normal males as soon as the normal oestrous cycle had been established, usually in 30 to 50 days. No initial resorption was allowed but the test material was fed within five days of the finding of sperm. Wheat-germ oil was used as a standard source of vitamin E for comparison with the prunes. The simple "all or none" criterion of production of a live litter was used for evaluation of vitamin E in the substance fed. This is, according to Mason (1942), still probably as good as any other method suggested.

The particular sample of wheat-germ oil used had borderline vitamin E content in one gram as demonstrated by the delivery of normal young by four out of five females given that amount. The prune-pit oil had similar value in 2.7 grams since three out of four matings resulted in normal litters when the pit oil was given. The prune pulp was found to have

little vitamin E activity. From 10 to 30 grams were given after mating to eight rats with only two litters resulting. An ether extract of 100 grams of prune pulp, however, provided enough vitamin E to allow one female to produce and suckle a normal litter. According to Evans, Emerson, and Emerson (1936) three mgm. of alpha tocopherol is sufficient to produce litters by vitamin E-deficient females on a similar diet to that used in this experiment; this is confirmed by Gottlieb, Quackenbush, and Steenbock (1943). It might be tentatively assumed then that 2.7 grams of prune-pit oil or 100 grams of prune pulp contain the equivalent of three mgm. of alpha tocopherol.

SUMMARY

French prunes of four crops were examined for certain vitamins. The conditions which were varied were crop year, size of fruit, and storage, processed and unprocessed and sprayed with prune pit oil or not so treated, raw or cooked.

A pleasant light oil which was extracted from the cracked pits and kernels was found to contain 40 to 50 U. S. P. units of vitamin A per gram and to make an acceptable glossy coating for dried prunes. This coating not only prevented "sugaring" of the surface during storage but also prevented insect infestation. It had no effect upon vitamin retention.

The vitamin A value of prune flesh, skins, paste, and water-extracted prune pulp was found by biological method to be 23 to 34 I. U. per gram of dry matter or 16 to 27 per gram of the usual market prune with 20 to 30 per cent moisture.

The thiamin content of four lots of 1939 dehydrated prunes stored 10 months and with various pretreatments was found by biological assay to be close to 0.5 mgm. per 100 gm. of dry matter or 0.3 to 0.4 mgm. per 100 gm. of ordinary market prunes. This is of the same order but greater than the value obtained by similar methods for sun-dried 1933 prunes.

As determined by biological method, the riboflavin of the dehydrated 1938 crop was somewhat larger than that of the 1939 crop and both were nearly double that of the sun-dried 1933 samples and both sun-dried and dehydrated 1942 samples. The values ranged from 0.7 to 1.8 mgm. per 100 gm. of dry matter or 0.5 to 1.3 mgm. per 100 gm. of market prune flesh.

By the microbiological method fresh and dehydrated prunes of the 1941 crop were found to have only 0.16 mgm. of riboflavin per 100 gm. of dry matter or about one-sixth the usual amount found by biological means. New determinations by both methods on fresh, dehydrated, and sun-dried 1942 prunes and upon recovery of added riboflavin indicated that the usual extractions for microbiological vitamin assays are unsatisfactory and unreliable when applied to prunes. New fluorimetric technique, however, yielded values similar to those obtained by rat-growth assay.

The pyridoxine (vitamin B_6) determined by rat assays in these prunes was somewhat lowered by cooking but not by storage. There were 0.6 to 1.5 mgm. per 100 gm. of dry matter or 0.4 to 1.2 mgm. per 100 gm. of market prunes.

Microbiological and biological assay of 1942 prunes revealed the same discrepancy for pantothenic acid as for riboflavin. The biological assay

indicated "filtrate-factor" activity equivalent to that of two to seven mgm. of pantothenic acid per 100 gm. of market prunes.

Nicotinic acid content of the 1942 prunes as determined by the microbiological method was 2.2 mgm. per 100 gm. of dry matter or 1.5 mgm. per 100 gm. of market prunes. This vitamin was not affected by the drying processes.

Vitamin E activity in amount equivalent to that of three mgm. of alpha tocopherol was found in the ether extract of 100 gm. of prune pulp or in 2.7 gm. of the prune-pit oil.

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COMPARATIVE STUDY OF STEAM AND HOT WATER BLANCHING [1]

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In many procedures for the processing of fruits and vegetables both commercially and in the home, the products first receive a blanching treatment, i.e., heating in a moist atmosphere to inactivate natural enzymes. This inhibits chemical changes which cause deterioration of palatability, appearance, and nutritional value. Thus, discolorations of diced and macerated fruits and vegetables and loss of labile nutrients (principally carotene and ascorbic acid) may be observed, if the materials have not been blanched. These undesirable changes frequently occur in a very short time, measurable in minutes.

Two procedures are used for the blanching of fruits and vegetables, one involving the use of steam and the other, hot water. The purpose of the present study was to determine which procedure is more desirable for routine use, particularly in home canning.

EXPERIMENTAL PROCEDURE

The test material used in this study was green snap beans obtained from a local farm. The blanching operation was effected within one and a half to two hours after the beans were picked.

Preparation of Beans: The beans were cut into pieces approximately one inch in length and the ends discarded; 218 grams of the cut beans (equivalent to one pint), were then rinsed in cold running water and immediately subjected to the blanching treatments. The blanched beans in all cases weighed the same as the raw product.

Steam Blanching Procedure: Sufficient water (75 c.c.) to cover the bottom of a pan was brought to a boil. The beans were suspended in a wire basket above the level of the boiling water. Care was taken to prevent direct contact between the water and the beans. A lid was immediately placed on the pan and the blanching time recorded. At exactly the end of specified periods of 1, 3, 5, and 10 minutes, the beans were cooled under cold running water followed by a distilled water rinse. The beans were then subjected to a variety of tests in order to determine the efficiency of the blanching procedure in inactivating the enzymes originally present.

Hot Water Blanching Procedure: The beans were prepared as described in the section above. In this case, however, they were added directly to 400 c.c. of boiling water, an amount sufficient to cover one pint of beans. At the end of each of the specified blanching periods the water was drawn off and the beans immediately cooled as described above.

During the steam blanching the beans were subjected to steam vapor at 100°C.(212°F.), though the temperature rise within the beans them-

[1] This study is one of a series, sponsored by the Westinghouse Electric and Manufacturing Company, Mansfield, Ohio.

selves was gradual. During the hot water blanching the addition of the beans directly to the boiling water caused an appreciable reduction of the water temperature, followed by a gradual return to the boiling point. Thus, in the case of the one-minute hot water blanch the average temperature of the water was only 82° C. (180° F.). In the tests with longer heating periods an average temperature of approximately 95°C. (203°F.) was maintained.

TABLE 1

Enzymic Tests Conducted on Green Beans

Tests for	Procedure
Monophenoloxidase [1]	5 c.c. of extract + 0.5 c.c. of 1% phenol
Polyphenoloxidase [1]	5 c.c. of extract + 0.5 c.c. of 1% catechol 5 c.c. of extract + 0.5 c.c. of 1% pyrogallol
Tyrosinase [2]	5 c.c. of extract + 5 c.c. of saturated solution of tyrosine, aerated at 30°C. (86°F.) 24 hr.
Indophenol oxidase [1]	5 c.c. of extract + 0.25 c.c. of 1% naphthol + 0.25 c.c. of 1% p-phenylenediamine + 0.25 c.c. of 10% Na_2CO_3
Peroxidase [1]	5 c.c. of extract + 1 c.c. of 4% benzidine + 1 c.c. of glacial acetic acid + 1 c.c. of 3% H_2O_2
Catalase [1]	Potassium permanganate titration of residual H_2O_2
Ascorbic acid oxidase [3]	Photometric determination of reduced, dehydro, and total ascorbic acid after maceration and incubation of samples

[1] The details of these tests are described by Hawk and Bergeim (1937). [2] Test based upon the properties of tyrosinase described by Sumner and Somers (1943). [3] Details of this test are given in the text.

CHEMICAL TESTS

In testing for enzymes other than ascorbic acid oxidase in the samples (Table 1), 150-gram quantities of the raw and blanched beans were homogenized for one minute in a Waring Blendor [2] with 150 c.c. of water under carbon dioxide. The suspension was diluted 1 to 2.5 (1 + 1.5) with distilled water, allowed to stand at room temperature for 10 minutes, and then filtered through cheesecloth. The filtrate at its natural pH of 5.8 was then analyzed.

Negative qualitative tests were obtained for monophenoloxidase, polyphenoloxidase, and tyrosinase in the raw as well as blanched beans, indicating the absence of these enzymes. The tests for indophenol oxidase and for peroxidase were carried out on a semiquantitative basis; the reagents were prepared as indicated (Table 1) and standard quantities of the test solutions and reagents were allowed to react under standardized conditions. The color intensities of the reaction products, however, were estimated only by visual comparisons. The catalase tests were conducted on a quantitative basis so that specific figures were obtained as an index of the concentration of this enzyme before and after blanching.

The results suggest that indophenol oxidase and catalase in green beans are more susceptible to heat inactivation than peroxidase (Table 2). This is in agreement with the report by Proctor (1942). The one-minute hot

[2] Obtained from the Waring Corporation, New York City.

water blanch almost, but not completely, destroyed the first two enzymes. However, approximately one-half of the peroxidase was still active. Extending the hot water blanching period to three to 10 minutes resulted in complete inactivation of these enzymes. A steam blanch of one minute's duration was not as effective as the hot water treatment in destroying these enzymes, since strongly positive tests for the indophenol oxidase, peroxidase, and catalase were still obtained in testing the extract. However, extending the steam blanching period to three to 10 minutes resulted in complete inactivation of these enzymes.

TABLE 2

Influence of Various Blanching Procedures Upon Enzymes in Green Beans

Sample	Experiment	Enzyme test		
		Relative color intensities[1]		H_2O_2 decomposition[2]
		Indophenol oxidase	Peroxidase	Catalase
				pct.
A	Raw beans, untreated	++++	++++	78
B	Steam blanch, 1 min.	+	+++	52
C	Steam blanch, 3 min.	—	—	7
D	Steam blanch, 5 min.	—	—	0
E	Steam blanch, 10 min.	—	—	0
F	Hot water blanch, 1 min.	±	++	4
G	Hot water blanch, 3 min.	—	—	6
H	Hot water blanch, 5 min.	—	—	0
I	Hot water blanch, 10 min.	—	—	0

[1] Semiquantitative estimations (scale of readings — to ++++). [2] To 10 c.c. of extract, representing two gm. of fresh or blanched green beans, 90 c.c. of M/150 H_2O_2 + 10 c.c. of buffer (pH 7.0) were added. The catalase, which may have been present, was allowed to act for 10 minutes. Decomposed H_2O_2 gives an index of catalase activity, Hawk and Bergeim (1937).

The results of various blanching methods upon ascorbic acid oxidase in the beans are presented (Table 3). The initial tests were conducted on samples which had been boiled with an equal weight of six-per cent metaphosphoric acid under nitrogen and then blended for one minute under nitrogen. This insured complete inactivation of the enzyme, as shown by Hochberg, Melnick, and Oser (1943). Other aliquots of the beans were blended with an equal weight of water under nitrogen for one minute and then incubated at 37°C.(98.6°F.) for two hours at their natural pH of 5.8. The suspensions were exposed to air in the incubator and were stirred every 10 minutes. The blending of the material with water and subsequent incubation of the sample, as indicated above, are favorable for the liberation of ascorbic acid oxidase and its activity.

Ascorbic acid oxidase catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. The latter is biologically active and should be determined in any investigation of vitamin C loss. Accordingly, the photometric method of Hochberg, Melnick, and Oser (1943) for the determination of both reduced and total ascorbic acid was employed in the present study; values for dehydroascorbic acid were obtained by difference.

The results (Table 3) indicate that ascorbic acid is very rapidly oxidized in the raw beans to dehydroascorbic acid during incubation and that

a significant quantity is oxidized beyond that stage. Steam blanching for one minute appeared to be ineffective in destroying the ascorbic acid oxidase. In the three-minute blanched sample no loss of total ascorbic acid was noted although an appreciable conversion of reduced to dehydroascorbic acid took place. Steam blanching for five or 10 minutes completely inactivated the enzyme.

TABLE 3

Influence of Various Blanching Procedures Upon Ascorbic Acid Oxidase in Green Beans

Sample	Experiment	Ascorbic acid values (mgm. per 100 gm.)					
		Initial tests			After incubation[2]		
		R[1]	D[1]	T[1]	R	D	T
A	Raw beans, untreated	18.2	4.3	22.5	1.2	16.0	17.2
B	Steam blanch, 1 min.	17.1	5.4	22.5	0.0	16.3	16.3
C	Steam blanch, 3 min.	17.1	5.8	22.9	10.8	12.2	23.0
D	Steam blanch, 5 min.	18.5	3.4	21.9	19.1	4.6	23.7
E	Steam blanch, 10 min.	18.7	3.1	21.8	17.5	4.6	22.1
F	Hot water blanch, 1 min.	18.4	4.0	22.4	9.1	6.8	15.9
G	Hot water blanch, 3 min.	17.1	3.6	20.7	15.5	2.7	18.2
H	Hot water blanch, 5 min.	15.3	3.7	19.7	15.0	4.8	19.8
I	Hot water blanch, 10 min.	15.1	3.1	18.2	14.1	4.4	18.5

[1] R = reduced ascorbic acid; D = dehydroascorbic acid; T = total ascorbic acid. [2] The beans were first blended under nitrogen with an equal weight of water and each suspension then stored at its natural pH of 5.8, for two hours at 37°C.(98.6°F.) under air; the suspensions were occasionally stirred under standardized conditions.

In the sample subjected to hot water blanching for one minute, the same extent of irreversible oxidation of ascorbic acid (beyond the dehydro state) was observed as in steam blanching for the same period of time. However, in this case more of the ascorbic acid remained in the reduced form. This indicates that after the short period of steam blanching more ascorbic acid was oxidized during the incubation. Some loss in total ascorbic acid was also noted in the sample which received the three-minute hot water treatment. Blanching for longer periods of time, five and 10 minutes, completely inactivated the ascorbic acid oxidase.

Results of the ascorbic acid study confirm those presented (Table 2) and demonstrate that hot water blanching over a very short period more effectively destroys the enzymes than the steam treatment. However, in this case also, the five-minute steam blanch was adequate for complete inactivation of the ascorbic acid oxidase.

The data (Table 3) were recalculated in order to show to better advantage the extent of oxidation of ascorbic acid during the incubation; the results are presented (Table 4). In this latter table are shown the percentage of the initial ascorbic acid converted to the dehydro form and that oxidized beyond this stage. These data demonstrate conclusively the greater effectiveness of hot water blanching in destroying the ascorbic acid oxidase but they also show that steam blanching for five minutes is completely effective. The significance of the small values for enzymic oxidation of ascorbic acid to the dehydro form (six or seven per cent) (Table 4) are questionable since dehydroascorbic acid is calculated from the difference between total ascorbic acid and reduced ascorbic acid. Thus,

errors in the analyses of only three per cent in opposite directions could account for the apparent six or seven per cent formation of dehydroascorbic acid. This conclusion is borne out by the fact that in both procedures the values for the 10-minute blanchings were not less than the values obtained for the five-minute heated samples.

In addition to the above results, other factors must be considered before a preference is indicated for any particular blanching procedure. The difference in effectiveness between these two procedures in destroying the enzymes present in the green beans favors hot water blanching. However, steam blanching for a period of five minutes is also satisfactory for complete inactivation of the natural enzymes.

TABLE 4

Influence of Various Blanching Procedures Upon Subsequent Oxidation of Ascorbic Acid

Sample	Experiment	Oxidation of ascorbic acid		
		Remaining in dehydro form [1]	Beyond dehydro stage [2]	Total [3]
		pct.	*pct.*	*pct.*
A	Raw beans, untreated	52	24	76
B	Steam blanch, 1 min.	48	28	76
C	Steam blanch, 3 min.	28	0	28
D	Steam blanch, 5 min.	6(?)	0	6(?)
E	Steam blanch, 10 min.	7(?)	0	7(?)
F	Hot water blanch, 1 min.	13	29	42
G	Hot water blanch, 3 min.	0	12	12
H	Hot water blanch, 5 min.	6(?)	0	6(?)
I	Hot water blanch, 10 min.	7(?)	0	7(?)

[1] Represents the percentage of the total initial ascorbic acid, found after incubation in the dehydro form, over and above the dehydroascorbic acid initially present. [2] Represents the percentage of total initial ascorbic acid, which has been oxidized beyond the dehydro stage and no longer possesses biological activity. [3] Represents the percentage of total initial ascorbic acid, which has been oxidized to or beyond the dehydro stage. The remaining ascorbic acid (100 minus the percentage in this column) represents the sum of the initial dehydroascorbic and residual reduced ascorbic acid, i. e., ascorbic acid which has not undergone any oxidation during the test incubation period.

Another factor of importance in a critical evaluation of blanching procedures is the degree of loss of the water-soluble nutrients. During the hot water blanch an appreciable loss of ascorbic acid occurred; the loss during the steam blanching was insignificant. This is apparent from the data on total ascorbic acid (Table 3). Analyses of the residual "blanching" waters in each case indicated the losses to be due to solution of the vitamin and not to heat destruction.

The report by Adam and Horner (1941), evaluating hot water and steam blanching with respect to retention of nutrients, also includes a study of vitamin C loss. Unfortunately no attempt was made to include dehydroascorbic acid so that their interpretation of the data with respect to true ascorbic acid loss is open to question. Likewise, in the report by Farrell and Fellers (1942) on the fate of ascorbic acid in blanched and unblanched beans, the very large loss (75 per cent) of the vitamin in the latter, caused by lowering the temperature of the beans to —20°C. (—4°F.), is undoubtedly in error. In this study also no attempt was made

to determine dehydroascorbic acid. Only in investigations involving prompt and complete inactivation of the ascorbic acid oxidase in vegetables prior to the rupturing of cells and in which the emphasis is on relative values, can dehydroascorbic acid be disregarded, Oser, Melnick, and Oser (1943).

In the report just cited the influence of various cooking procedures on the retention of vitamins and minerals in vegetables was investigated. It was shown that the other water-soluble nutrients in foods are lost approximately to the same degree as ascorbic acid when cooking water is discarded. Thus, it may be assumed that the progressive loss in total ascorbic acid in the beans subjected to hot water blanching reflects the over-all loss of other water-soluble nutrients such as thiamin, nicotinic acid, riboflavin, etc.

Considering both aspects, destruction of the enzymes present and retention of the essential nutrients, the conclusion is reached that steam blanching, although for a somewhat longer period of time, is superior to hot water blanching. In the present study, duplicating home canning of green beans, a steam blanch of at least five minutes is regarded as the preferred procedure.

SUMMARY

A comparative study was conducted of two blanching procedures as used in home canning. The influence of hot water (100°C.) and steam (100°C.) blanching of green snap beans upon the enzymes, indophenol oxidase, peroxidase, catalase, and ascorbic acid oxidase, was evaluated.

Hot water blanching of the beans was more effective in destroying the natural enzymes in this vegetable, a three-minute exposure being almost completely effective in this respect.

Five minutes' steam blanching is necessary for complete inactivation of the enzymes in green snap beans.

The disadvantage of hot water blanching resides in the fact that the soluble nutrients of the vegetables, as typified by ascorbic acid, are lost to an appreciable extent in the blanching water.

Loss of soluble constituents during steam blanching is not significant.

Since the difference between the two blanching procedures in completely destroying the enzymes in the beans is not appreciable with respect to time, the steam blanching method is to be preferred.

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HEMOGLOBIN-REGENERATING PROPERTIES OF PRUNES[1]

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Prunes were found by Robscheit-Robbins and Whipple (1927) to be remarkably effective for blood regeneration in dogs made anemic by hemorrhage. They found apricots and peaches comparable with muscle meat and second only to liver in earlier experiments, and later concluded that prunes are nearly as efficient as these two fruits. They ascribed this property of the fruits to mineral constituents other than iron since other fruits equally rich in iron, for example raspberries, were without hemoglobin-producing value.

Since the amount and availability of the iron and copper contents of foods are now known to vary greatly and the hemoglobin-regenerating properties of the foods to be dependent upon these elements, it seemed desirable to determine directly the anti-anemia value of prunes as well as their ash, iron, and copper contents. The value of prune flesh, skins, and pits might vary and accordingly vary the value of large and small fruit. Culls or very small fruit, which may be used whole to make a paste for use as animal feed, were of particular interest since the large proportion of pits and skin might be expected to alter the mineral as well as other composition of the product.

Accordingly, total ash as well as iron, copper, and other inorganic elements were determined in two different-sized lots of 1938 prunes; ash and iron analyses of several sizes of 1939 prunes and pastes and bio-assay were carried out to find the availability of iron and copper of several of these products.

ASHING METHOD

Two methods of ashing were compared[3]—a modification of a quick method at high temperature with addition of rare earth salts, first proposed by Walters (1930) for ashing flour, and the normal ashing method, Association of Official Agricultural Chemists (1935). The prune flesh was cut into small pieces with a silver knife and dried in lots of 15 to 20 grams in shallow, covered aluminum dishes. The drying was continued to "constant weight," which required five to seven days at 70 to 75°C. (158 to 167°F.).

The modified or quick method of ashing involved treating five-gram samples with 20 ml. of cerium nitrate solution [1.8915 gm. Ce $(NO_3)_6$·

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[3] The ashing comparison and part of the ash analysis was done by Y. J. Minassian.

$12H_2O$ per liter of 40-per cent alcohol] for one hour in the cold form, stirring every 10 minutes. The alcohol was then burned off and the crucibles were placed in a muffle furnace for 20 minutes at 790°C. Three blanks were run to determine the weight of cerium oxide deposited, and the average value thus obtained was used in calculating the percentage of ash.

For the "normal" ashing method five-gram samples were heated at 580°C. for 24 hours, a few drops of concentrated nitric acid added, and the ash then heated in the muffle furnace to constant weight.

The normal method in four determinations and the quick method in five gave practically the same mean for per cent of ash. The time of the analyst required for the operation was, however, about the same in the two, but the use of cerium nitrate appeared to increase the precision. If

TABLE 1

Ash Analysis of Prune Flesh[1]

Elements	Size 20-30,[2] 1938 crop		Size 120, 1938 crop	
	Normal ash	$Ce(NO_3)_6$ ash	Normal ash	$Ce(NO_3)_6$ ash
Total ash	1.95	1.95	3.25	3.21
Ca	0.069	0.069	0.109	0.109
Mg	0.074	.....	0.124	.....
P	0.136	0.089	0.176	0.143
Cu	0.00085	0.00082	0.00101	0.00107
Fe	0.0062	.....	0.0173	.....

[1] Expressed in per cent of dry weight. [2] Number of prunes per pound.

the dried samples were ground to a fine powder, weighed, and the cerium nitrate added to this sample, better incineration resulted. A homogeneous purée, obtained by adding a measured quantity of water to the sample and then subdividing it in a blender, is still better suited for ashing by the cerium nitrate method. The present official method, Assoc. Off. Agr. Chem. (1940), involves similar use of magnesium acetate.

ASH ANALYSIS

The calcium and magnesium were determined by "Official Methods" (1935) in the ashes obtained by the two methods; phosphorus by the method of Fiske and Subbarow (1925); iron by that of Elvehjem (1930), copper by that of Elvehjem and Lindow (1929). It will be noted (Table 1) that the only significant difference between the ashes obtained by these two methods lies in the apparent loss of P in the cerium nitrate ash.

The very small fruit had a significantly greater content of all ash elements, indicating concentration of these elements in the skin.

The 1938 samples (Table 1) used for this analysis were not from the same lot later utilized in the blood regeneration studies but the 1939 samples (Table 2) represented those used in rat experiments (Table 4). Several samples of the 1939 crop were analyzed for ash and iron, the latter by the colorimetric orthophenanthrolene method of Saywell and

Cunningham (1937) (Table 2). It was interesting to note that the pits were relatively poor in mineral constituents compared with the flesh, and the whole ground fruit was therefore lower in iron than the flesh.

A paste [4] made of whole cull fruit was found to contain nearly 10 times as much iron as the flesh of large prunes. Three samples, taken at progressively advanced stages of the crushing and grinding operation, contained 1.76, 1.86, and 2.07 ash per cent of wet weight and 0.31, 0.59, and 0.91 per cent of iron in these ashes. Apparently iron from the grinding plates was taken up by the paste.

TABLE 2

Ash and Iron in Prunes, 1939 Crop

Fruit samples	Water	Ash (dry weight)	Iron		
			Dry weight	Ash	Fresh weight
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
50-60 size, flesh only	26.5	2.25	.0031	0.14	.0023
Whole prunes	22.0	1.95	.0025	0.13	.0020
100-120 size, flesh only	28.7	2.83	.0044	0.15	.0031
Pits only	10.2	0.81	.0077	0.09	.0006
Whole prunes	25.0	2.17	.0030	0.14	.0022
Prune paste	23.0	2.25	.0320	1.42	.0260
Prune paste, no pits	35.8	2.38	.0374	1.57	.0270

This paste, which was used in some of the anti-anemic tests with rats and later in a dog-feeding experiment, was compared with a similar paste containing no pits (Table 3).

TABLE 3

Composition of Prune Pastes

Paste	Ether extract	Protein	Ash		Iron		
			Wet	Dry	Of ash	Wet	Dry
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Whole prunes (23% water)	.37	2.53	1.83	2.25	1.42	.026	.032
Flesh only (35.8% water)	.33	1.81	1.75	2.38	1.57	.027	.037

So far as these data go it may be assumed that the paste containing pits is not inferior to that without pits but, as will be noted later, the whole prune iron was less valuable biologically. No evidence of toxicity from the presence of amygdalin in the kernel was noted in the animals which were fed fairly large amounts of the whole prune paste, 20 per cent of the whole food given, for example, in the case of young dogs. It is plain that toxic breakdown of the cyanide-containing compound of the kernels does not take place in the dried fruit.

The range of iron content in these 1939 samples is in most cases quite like that found by Saywell, Dietz, and Robertson (1934) by the Stugart (1931) method, except for the very small fruit of the 1938 crop which

[4] These pastes were supplied by Rosenberg Brothers and Company of San Francisco.

was not used in the rat experiments. The preparation of samples for analysis may have a significant effect upon the iron content, however, since surface dust is likely to be rich in iron, and treatment of trees with various iron and copper-rich sprays and fertilizers may affect the composition of the fruit. We have no record of the exact origin of the 1938 samples. Saywell *et al.* (1934), however, used fruit known to be free from treatment with iron or copper salts. The copper content of the two 1938 prune samples analyzed in this study was about three times that found by Saywell and co-workers.

TABLE 4

Hemoglobin Regeneration in Anemic Rats Fed Prune Products

Daily iron intake	Copper supplement	Source of iron	Number of rats	Hemoglobin (gm./100 gm. blood) gain in 5 weeks	Crop year and size of prunes
mgm.	*mgm.*				
.05	.05	Iron as $FeCl_3$	4	3.7	.....
.05	None	Prune-flesh ash, 36 mgm.	4	1.6	1938, 50-60
.10	.05	Iron as $FeCl_3$	4	4.7	.....
.10	.05	Prune flesh, 2.2 gm.	5	4.4	1938, 50-60
.10	None	Prune flesh, 2.2 gm.	10	2.4	1938, 50-60
.10	None	Prune flesh, 4.0 gm.	7	2.3	1939, 50-60
.20	.05	Prune flesh, 2.3 gm.			
.00	.00	iron as $FeCl_3$, 0.1 mgm.	6	5.9	1938, 50-60
		None	17	—1.8 (6 died)	.....
.15	.05	Iron as $FeCl_3$	9	7.2	.....
.15	None	Prune flesh ash, 100 mgm.	3	6.2	1939, 100-120
.15	.05	Prune flesh ash, 100 mgm.	10	6.6	1939, 100-120
.15	None	Prune flesh, 5 gm.	10	7.0	1939, 100-120
.15	None	Prune paste, 0.6 gm.	8	6.5	1939, 100-120
.15	.05	Whole-prune ash, 107 mgm.	8	6.6	1939, 100-120
.15	None	Whole-prune paste, 0.6 gm.	9	6.7	1939, 100-120
.00	.05	None	9	—2.0 (3 died)	.....
.30	.05	Iron as $FeCl_3$	10	8.5	.....
.30	.05	Whole-prune ash	10	6.6	1939, 50-60
.30	.05	Prune-flesh ash	10	8.6	1939, 50-60

BIOLOGICAL EXPERIMENTS

The method used to determine the availability of the iron and copper of prunes for hemoglobin production was the well-known nutritional-anemia technique with rats. Young weanling rats were placed on a diet of whole dried milk, on which regime they grew normally but gradually became depleted of the mineral elements required for hemoglobin production. After three to six weeks the level of hemoglobin, determined by the Newcomer method on tail blood, usually fell to five per cent or less. The animals were then grouped as to sex and litter so that comparable animals were given the various test doses for the following five to eight weeks. Altogether 225 rats were used in five experiments. Four of these experiments, summarized (Table 4), dealt with the value of prunes and prune ash as source of iron and copper fed at different levels. The fifth experiment was

concerned with the curious depigmentation of fur which was noted in this study, as it has been previously by others.

The difference in utilization of iron between male and female anemic rats, shown by Smith and Otis (1937a), was taken into account in the grouping of the animals but was not found to be consistent.

The prunes and ashes were fed at four levels of iron intake, 0.05, 0.10, 0.15, and 0.30 mgm. daily, and the ash solutions were given separately from the basal diet and were eaten promptly and completely by the rats. In all cases the control animals were given 0.05 mgm. of copper and 0.04 mgm. of manganese daily in order to insure full utilization of the iron fed as $FeCl_3$. Glass-tipped water bottles, glass food cups, and well-tinned wire cages were used.

The hemoglobin regeneration of the rats fed the various levels of iron as pure ferric chloride was quite similar to, but slightly less than, those found by Smith and Otis (1937b) even though the hemoglobin levels achieved on four or five weeks' depletion were higher than those ordinarily found. The administration of 0.05 mgm. of copper throughout the depletion period and to the negative groups was not observed to affect the speed of depletion and only slightly the final hemoglobin level, but the weight gain was increased when copper was fed.

In a series on 0.30 mgm. iron level, fresh liquid milk and dry whole milk as the basal diets were given to parallel groups. There was little difference in the onset of severity of the anemia, but the 23 rats fed on liquid milk in 30 days attained an average weight of 98 grams and 4.7 per cent hemoglobin, while 25 litter mates on dried whole milk attained 119 grams and 5.4 per cent. The responses of these two groups to the supplements were alike. Dried milk is more satisfactory both as to use and control of composition than is liquid milk and ensures more growth and fewer deaths during the depletion period. Since the response to equal amounts of iron appeared to be substantially the same in the rats fed either type of milk, the dried milk was adopted for use in later experiments.

The ash of prunes (120 size) of the 1938 crop was used in a series at the level of 0.05 mgm. of iron and was found not to be as useful for hemoglobin regeneration as the same amount of iron as $FeCl_3$. No copper was given with the prune-ash supplement and this may well account for the failure. The group given ash of prune pits at this level also made poor hemoglobin regeneration.

In the series at the 0.10 mgm. iron level prune flesh (50-60 size, 1938 crop) was fed as source of iron with 0.05 mgm. of copper and the same amount without the copper. In the latter case the availability of the iron was 50 per cent but with the copper, 93 per cent. In a later experiment the value of 50-60 size prunes (1939 crop) without copper was found to be just the same as for the 1938 samples. The limiting factor in the utilization of the prune iron at this level appeared to be the accompanying copper.

In another experiment both ash and prunes were fed as the source of iron at the 0.15 mgm. level with success. These were 100-120 size prunes (1939 crop) ashed in the laboratory and also crushed to a paste,[4] whole

or with pits removed. The whole-prune paste and its ash, flesh paste and its ash, and the unground flesh were found to be equally effective as sources of iron at this level. The pit-ash iron was less available.

The substantial equivalence of the prune products and their ashes for hemoglobin production indicates the excellent availability of the iron of the fruit. In this respect prunes are like legumes and molasses, Sheets and Ward (1940), and unlike spinach and other leaves, Sherman, Elvehjem, and Hart (1934). Our effort to determine the ionizable iron of prunes by the dipyridyl method of Hill (1930) was unsuccessful because of the deep coloring of the extracts obtained with dilute acid and alcohol. The dark solutions were cleared with charcoal but the filtrates apparently contained no ferric iron. Either no iron was extracted or more probably it was adsorbed on the charcoal. Shackleton and McCance (1936), however, reported 3.2 mgm. per cent total iron in "dry prunes" and found 72 per cent of it available, as determined by the dipyridyl method.

The whole question of the reliability of any chemical method of prediction of biological value of food iron is unsettled. Some investigators are satisfied with the agreement between chemical and biological tests of the value of foods as sources of iron for hemoglobin production and others are not. The relative availabilities found by different investigators for the same type foods and by the two methods are in many cases not in satisfactory agreement.

Examination of the data (Table 4) leads to the conclusion that both the whole ground prunes and their ash and pit ash provide somewhat less valuable iron than the ground prune flesh and its ash. At the 0.3 mgm. iron level, for example, the flesh ash gave 8.6 and the whole prune ash 6.6 per cent increase of hemoglobin in parallel groups. This may be due either to greater concentration of copper in the flesh than in the pits or to decrease in solubility of the ash elements in the presence of silica or other indigestible constituents of the pits. In an earlier biological study with anemic rats, Hanning (1934) noted an increase of 2.0 gm. of hemoglobin per 100 gm. blood when prunes were fed to supply 0.1 mgm. of iron and 0.006 mgm. of copper daily. This agrees closely with the effect of the same amount of iron in prunes without supplemental copper used in this experiment (Table 4). The deficiency appeared to be due to lack of copper in the prunes, according to Hanning. The iron content in proportion to copper of the prunes used must have been excessive (23 and 0.0013 mgm. per cent) if 0.441 gm. dried prunes furnished 0.1 mgm. iron and 0.006 mgm. copper.

At the higher levels of prune and prune ash intakes, that is, daily amounts yielding 0.15 mgm. of iron or more, the amount of copper present appeared sufficient to insure maximum iron utilization, but at lower levels the copper appeared to be the limiting factor of the antianemic value of the samples. Thus the ashes at 0.05 mgm. level without copper had low availability and the prune flesh at 0.10 mgm. iron level had 50 per cent without copper and 93 per cent with copper. At 0.15, 0.20, and 0.30 mgm.

iron levels the iron of ash, flesh, and whole fruit was 74 to 97 per cent available.

GREYING OF FUR OF ANEMIC RATS

Greying of the fur of black and piebald rats was noted after three to six weeks on the milk diet used to produce low hemoglobin levels for the study of the response of these animals to prunes. This has been observed frequently by others. Keil and Nelson (1931) first reported the depigmentation as caused by milk anemia and as not cured by administration of iron but only by iron and copper together. Gorter (1935, 1939) did not investigate the blood picture of his animals but obtained greying in rats and cats which responded to copper only and not to zinc; Stirn, Elvehjem, and Hart (1935) found the grey and scanty coats of zinc-deficient rats returned to normal by zinc feeding. The latter investigators also did not report on blood composition. Free (1940) examined the greying produced by milk diet in comparison with that due to "filtrate-factor" deficiency and found them specific and not amenable to interchanged treatment.

Of the first lot of 76 rats made anemic on milk diet it was noted that within five to eight weeks all dark-furred animals developed greying or rufous patches and in many cases lost the long guard hairs, presenting a mole-like appearance of the coat. In some cases the hair fell out, and bald or thinly covered patches developed. Some of these animals which were kept for four to five months on 0.05 mgm. of iron daily with hemoglobin level of six to nine per cent were given large doses of concentrated yeast extract, a treatment which had been found rapidly curative of the depigmentation produced by "filtrate-factor" deficiency. This deficiency syndrome was undoubtedly largely due to lack of pantothenic acid although other and still unidentified factors were probably involved. In the anemic grey rats the treatment was ineffective both as to hematopoiesis and the greying of fur. Persistent thymuses, mottled livers, and pitted kidneys were found on autopsy.

Another group of seven of these rats after four months on the same regime (0.05 mgm. iron, 0.05 mgm. copper, and 0.04 mgm. manganese daily) showed similar greying and loss of fur and low hemoglobin, six to eight per cent. They were then given 0.5 mgm. iron, 0.1 mgm. copper, and 0.04 mgm. manganese daily for five months. The hemoglobin level rapidly rose to 15 to 18 per cent but in only one case was there a return to normal pelage. Some improvement was noted in four and no change in two others. On autopsy there were found mottled pebbly livers and pitted kidneys, the pelvis being completely destroyed in two cases. The removal of the iron and copper deficiency did not, during this relatively long time, repair the hair and skin damage or prevent the liver and kidney changes. Since in an extensive experience with cure of pantothenic acid-deficient rats failure of re-pigmentation in such a period has not been observed, it would appear that delayed moult is not responsible.

A new lot of 26 rats from four litters was then placed on the milk diet and divided into four groups to receive daily supplements as follows: (1) 0.05 mgm. iron, 0.05 mgm. copper, 0.04 mgm. manganese; (2) 0.3 gm. concentrated yeast filtrate; (3) both supplements given Groups 1

and 2; (4) no supplement. There was no depletion period so that most of the rats were never severely depleted of iron reserves. After two months, when the hemoglobin levels varied from eight to 11 per cent, all groups had made nearly the same weight gains and all were equally grey.

Since it seemed possible that these animals might be suffering from B-vitamin deficiencies two out of each group were given further supplements of thiamin, riboflavin, and pyridoxine, 10 micrograms each per day for the following two months. No increase in rate of weight gain followed but there was a prompt rise in hemoglobin, averaging 3.4 per cent. Addi-

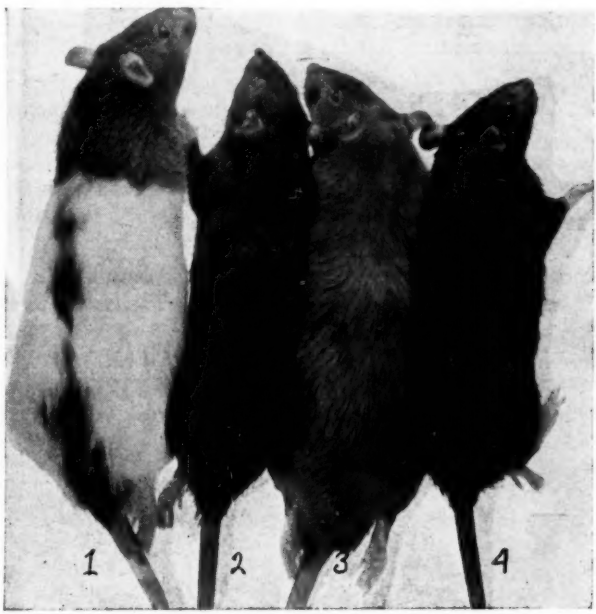

FIG. 1. Greying produced by nutritional anemia in the same period, three to four months, in Rats 1 and 3; and by pantothenic acid (filtrate-fraction) deficiency in Rat 4. Rat 2 was normal.

tional iron, 0.05 mgm. daily, brought about a rise of 2.1 per cent in another small group, but increase in the yeast filtrate to 1.2 gm. per day had no effect. The latter group and all others with no change in supplements made no gains or lost one to three per cent hemoglobin. There was little change in fur color in any of these rats, although as hemoglobin levels rose some re-pigmentation usually occurred.

Photographs of two of these animals, a control and a non-anemic, pantothenic acid-deficient rat, are shown (Fig. 1). It seemed clear that pantothenic acid and other substances found in the nonadsorbable fraction of the B complex are not concerned in hemoglobin production but that the adsorbable group may be. György, Robscheit-Robbins, and Whipple

(1938) found that riboflavin increased the hemoglobin-regenerating capacity of dogs. Similar activity was found in pyridoxine given to rabbits after venesection, Döllken (1940). Leverton and Marsh (1942) noted better retention of iron by young women eating diets generous in other nutrients, including the B vitamins, on the same low iron intake than others living on suboptimum diets.

SUMMARY

Several samples of California French prunes were ashed by two methods and analyzed for certain mineral constituents, particularly iron. Total ash and all elements determined were larger in the flesh of small than of large sized fruit, indicating mineral concentration in the skins. Whole fruit and pits were relatively poor in ash and in iron.

The regeneration of hemoglobin in rats made anemic by whole-milk diet was not as good at low levels of iron intake in animals given prunes as in those given inorganic iron and copper, but when small amounts of copper were given also, the prune iron became practically completely available. At the higher levels of iron intake the prune flesh and its ash were nearly equivalent to the inorganic iron but the whole prunes, including pits and their ash, were somewhat less effective.

It is concluded that prune iron is largely available for hemoglobin production but its usefulness is limited at lower levels of intake by the prune copper content.

The greying of the fur seen in the milk-fed rats was only partly remedied by iron and copper feeding as well as by pantothenic acid and unknowns in a concentrated yeast filtrate. Supplements of thiamin, pyridoxine, and riboflavin, however, improved hemoglobin regeneration and promoted some re-pigmentation.

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EFFECT OF VARIOUS HOME PRACTICES ON ASCORBIC ACID CONTENT OF CABBAGE[1]

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This study was undertaken in order to determine how the ascorbic acid content of cabbage was affected by different home procedures. There are many reports in the literature concerning the ascorbic acid content of cabbage, and several that deal with the effect of cooking and advance preparation on the amounts of ascorbic acid present.

Freshly harvested cabbage has been found to contain varying amounts of ascorbic acid. Burrell, Brown, and Ebright (1940) determined the amounts of ascorbic acid in 30 varieties and strains of cabbage, obtaining an average figure of 1.002 milligrams of ascorbic acid per gram of cabbage. The 10 highest varieties and strains gave an average of 1.529, while the average for the 10 lowest was 0.592. For small-headed cabbage Floyd and Fraps (1939) report an average value of 130 milligrams for 100 grams. Mayfield and Richardson (1940) found that Danish Ballhead cabbage, harvested in the latter part of October, contained 0.63 milligram of ascorbic acid per gram. Murphy (1942) determined the ascorbic acid content of four varieties of cabbage, which were all grown in four localities, and found that the values varied from approximately 22 to 138 milligrams per 100 grams. A range from 0.31 to 0.44 milligram per gram in nine samples of freshly cut cabbage was reported by Pederson, Mack, and Athawes (1939). Ranganathan (1935) obtained values of 132.6 milligrams per cent of ascorbic acid for the fresh inner leaves of cabbage, and 125.8 for the fresh outer leaves. Tressler, Mack, and King (1936) reported an average value of 0.31 milligram of ascorbic acid per gram for four varieties of cabbage which they studied. Gould, Tressler, and King (1936) determined the ascorbic acid content of three varieties of summer cabbage, and three of the fall type; the average values were 0.55 and 0.29 milligram per gram, respectively.

The loss of ascorbic acid on cooking cabbage has been of interest for several years, and in general, the losses reported are rather high. Delf (1918) heated cabbage at various temperatures and obtained a 70-per cent loss when it was cooked at 90 to 100°C. (194 to 212°F.) for 20 minutes. Eddy, Shelow, Pease, Richer, and Watkins (1923) stated that cooking cabbage by either the open-kettle method or the pressure-cooker process caused destruction of vitamin C which was slightly less than 95 per cent of the total. This amount represented the loss caused by heat and by extraction. Jarussowa (1935) found that approximately 80 per

[1] Work undertaken as part of the National Cooperative Experiment Station Project on "Conservation of Nutritive Values of Foods."

cent of the vitamin C remained in the cabbage and cooking water after cooking for one hour. Halliday and Noble (1936) boiled cabbage which was cut in small pieces in a large amount of water for 10 minutes and found that 69 per cent of the ascorbic acid was in the cooking water while none was destroyed. Burrell and Ebright (1940) reported losses for cooked shredded cabbage of 70 per cent when the cooking water was discarded and 55.5 per cent when the water was saved. Mayfield and Richardson (1940) obtained a loss of approximately 55.5 per cent on boiling cabbage. Gould, Tressler, and King (1936) boiled cabbage in a large amount of water and found that less ascorbic acid was retained in the cabbage as the boiling times were increased. Wellington and Tressler (1938) cooked cabbage cut into pieces of different size, by three methods, and found that the amount of ascorbic acid retained varied from 66 per cent when finely shredded cabbage was panned to 22 per cent when similar cabbage was boiled.

A few studies have been made of the effect on the ascorbic acid content of preparing cabbage for serving raw. Mayfield and Richardson (1940) obtained the same values for the ascorbic acid content of cole slaw tested immediately after preparation, and tested again after it had stood for one hour. Lampitt, Baker, and Parkinson (1942) stated that the concentration of ascorbic acid in shredded cabbage fell rapidly during the first 10 to 15 minutes, and that the value then obtained remained constant for 30 hours provided that the cabbage was kept cool. Pyke (1942) reported that there was no significant loss of ascorbic acid in cabbage shredded with a sharp knife during a subsequent three-hour period of standing.

This work was planned in order to determine the effect of several common procedures on the ascorbic acid content of cabbage. These included shredding cabbage and allowing it to stand for definite periods of time in contact with the air and covered with water; boiling cabbage in three different amounts of water and for three periods of time; cooking cabbage, holding the cooked product in the refrigerator, and reheating it after one and three days; and, incidentally, the effect of storing cabbage under different conditions.

EXPERIMENTAL PROCEDURE

Wisconsin Golden Acre, Racine Market, and Resistant Detroit varieties of cabbage, supplied by the Department of Horticulture, were used in this study. This cabbage was grown on prairie loam soil and was harvested on October eighth. In general, the heads were fairly small, although the weights ranged from 240 to 857 grams. The heads were not firm and the outside leaves were green. When the cabbage was harvested, 18 heads were taken for moisture and ascorbic acid determinations, and the rest were placed in 50-pound, tightly covered, lard cans. These were stored at the Horticulture Field Laboratory in a room where the temperature varied from —0.5 to 4°C.(31 to 39°F.). One can was brought to the Home Economics Laboratory, as needed, and kept in the refrigerator at 2 to 7.5°C.(35.6 to 45°F.).

For the determination of ascorbic acid the titration method of Tillmans, Hirsch, and Hirsch (1932), as modified by Bessey and King (1933)

and Bessey (1938), was used. Ten-gram samples of cabbage were weighed out in 20 ml. of three-per cent metaphosphoric acid. The samples were ground in the acid with sand; the mixtures were centrifuged and the supernatant liquids were filtered through cotton into volumetric flasks. Washing and re-extraction was carried out with four 15-ml. portions of metaphosphoric acid, and the extracts were made up to volume. Aliquots were titrated with solutions of 2,6-dichlorophenolindophenol, which were made up every five days and standardized daily with solutions of crystalline ascorbic acid.

Samples for the determination of ascorbic acid in raw cabbage were prepared by quartering each head of cabbage in the lot and using alternating lengthwise and crosswise sections from slices of each quarter until the desired weight was obtained. Portions of the core were not included since the cores were removed prior to cooking. In any one experiment on the effect of variations in cooking, or advance preparative procedure, one quarter of each cabbage was placed in the lot to be analyzed raw, and the others in three different lots to be used for the three variations. The resulting products were sampled at random.

Cabbage was prepared in the following ways to determine the effect of variation in procedure on the ascorbic acid content.

Variation in Amount of Water Used for Cooking: Four hundred-gram portions of approximately ¾-inch slices of Wisconsin Golden Acre cabbage were boiled in 200, 800, and 1,600 ml. of tap water in aluminum pans with fitted covers for seven minutes. All the cooked products were of good quality; the cabbage cooked in 1,600 ml. of water was slightly softer and greener than that cooked in 200 ml. of water. Details of the cooking procedure—regulation of the gas burners, control of the amounts of water, amounts of salt used, and draining and weighing of the cooked products—were carried out according to the mimeographed outline of Recommended Laboratory Procedures for Food Preparation and Food Preservation for the National Cooperative Experiment Station Project on "Conservation of Nutritive Values of Foods" in all of the experiments. Three extractions and determinations of ascorbic acid were made on the raw portion and on each of the cooked portions, and the experiment was repeated four times. Two aliquots of each cooking water were used for ascorbic acid determinations.

Variation in Time of Cooking: Four hundred-gram portions of sliced Wisconsin Golden Acre cabbage were boiled in 800-ml. volumes of water for 7-, 15-, and 25-minute periods. The seven-minute boiling period was optimum for the tender, green cabbage used. Three extractions were made on each sample.

Variation in Method of Boiling Cabbage to the Same Stage of "Doneness" Judged Subjectively: Four hundred grams of Resistant Detroit cabbage were boiled in 800 ml. of water for seven minutes in a covered aluminum pan. Two 400-gram portions were boiled in 800 and 1,600 ml. of water for five and one-half and eight and one-half minutes, respectively, in uncovered aluminum pans. Four extractions were made on each portion.

Variation in Time of Holding Cooked Cabbage Before Reheating: Three 400-gram portions of sliced Wisconsin Golden Acre cabbage were boiled in

800 ml. of water for seven minutes. One was sampled immediately while the others were allowed to cool for one hour and then held in covered containers in the refrigerator at 4 to 6°C.(39.2 to 42.8°F.) for 24 and 72 hours. After holding, they were boiled in 100 ml. of water for two minutes. Four extractions were made on each portion and the experiments were repeated four times.

Effect of Advance Preparation of Cabbage: Three hundred-gram portions of Racine Market cabbage were shredded with a stainless-steel knife. The slices were approximately $\frac{3}{16}$ of an inch in width; one portion was allowed to stand in contact with the air for one hour and two were covered with the tap water and allowed to stand for one- and three-hour periods. In addition two lots of cabbage purchased on the market were used in this portion of the study.

Effect of Storage: The ascorbic acid content of six Racine Market cabbages was determined at harvest and after one, two, and three months of storage. After storage in the Horticulture Field Laboratory, 16 heads of Resistant Detroit cabbage were divided into four lots. One lot was analyzed immediately; one was allowed to stand at room temperature of 25°C. (77°F.) for three days; one was kept in the refrigerator for four days; and the last was in the refrigerator for seven days. During this period the refrigerator temperature ranged from 3 to 8°C.(37.4 to 46.4°F.).

RESULTS AND DISCUSSION

The figures for the ascorbic acid content of cabbage are given in milligrams per gram as determined, unless otherwise specified, while the per cent values for retention, solution, and loss are calculated on the basis of the original raw weights.

At the time of harvest, Wisconsin Golden Acre, Racine Market, and Resistant Detroit cabbages contained, respectively, 0.42 ± 0.02, 0.47 ± 0.00, and 0.48 ± 0.01 milligram of ascorbic acid per gram. In general, these values are lower than others reported in the literature. However, they are higher than the figures obtained by Gould, Tressler, and King (1936) for three types of autumn cabbage and the average computed by Pederson, Mack, and Athawes (1939) from their ascorbic acid determinations on nine samples of freshly cut cabbage. Burrell, Brown, and Ebright (1940) reported a higher ascorbic acid content for Resistant Detroit cabbage than was found in this study.

The figures for the ascorbic acid content of cabbage after three periods of storage and after subsequent holding at two temperatures (Table 1) show that if the ascorbic acid content is calculated in milligrams per gram of dry weight, it is evident that no significant loss of ascorbic acid occurred during the first two months of storage or during holding in the refrigerator for a week. Losses occurred during the third month of storage and when cabbage was held subsequently at room temperature for three days. In the case of cabbage held at room temperature, the outer yellowed, wilted leaves were discarded before the determinations were made so the total loss of ascorbic acid was probably higher than these figures indicate. Mayfield and Richardson (1940) observed a decrease of about 25 per cent

TABLE 1

Ascorbic Acid Content of Three Varieties of Cabbage When Harvested and After Different Periods of Storage

Date analyzed	Variety of cabbage	Treatment	Number of determinations	Ascorbic acid mean and average deviation, fresh weight	Ascorbic acid mean and average deviation, dry weight
				mgm./gm.	*mgm./gm.*
10-8-42	Wisconsin Golden Acre	When harvested	6	.42±.02	5.8±0.2
11-6-42	Wisconsin Golden Acre	After 29 days' storage [1]	4	.38±.02	5.6±0.2
10-8-42	Racine Market	When harvested	6	.47±.00	6.4±0.1
11-9-42	Racine Market	After 32 days' storage [1]	6	.49±.02	7.3±0.2
12-9-42	Racine Market	After 62 days' storage [1]	6	.39±.02	6.2±0.3
1-9-43	Racine Market	After 93 days' storage [1]	6	.37±.01	5.8±0.2
10-8-42	Resistant Detroit	When harvested	6	.48±.01	6.4±0.1
12-4-42	Resistant Detroit	After 57 days' storage [1]	10	.42±.01	6.3±0.2
12-7-42	Resistant Detroit	After 57 days' storage and 3 days at room temperature [1]	12	.39±.01	5.5±0.2
12-8-42	Resistant Detroit	After 57 days' storage[1] and 4 days in refrigerator	12	.47±.01	6.2±0.1
12-11-42	Resistant Detroit	After 57 days' storage[1] and 4 days in refrigerator	12	.45±.01	6.2±0.2

[1] In tightly covered 50-pound lard cans at the Horticulture Field Laboratory.

TABLE 2

Effect of Variations in Amounts of Water Used in Boiling on Ascorbic Acid Content of Cabbage [1]

Date	Raw cabbage		400 gm. cabbage in 200 ml. water, boiled for 7 min.				400 gm. cabbage in 800 ml. water, boiled for 7 min.				400 gm. cabbage in 1,600 ml. water, boiled for 7 min.			
	Moisture mean and average deviation	Ascorbic acid mean and average deviation	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost
	pct.	*mgm./gm.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*
10-23-42	92.9±0.1	.40±.01	.34±.01	78	12	10	.22±.00	55	25	20	.21±.01	50	23	27
10-26-42	92.9±0.1	.41±.00	.34±.01	75	14	11	.26±.03	66	26	8	.21±.01	51	18	31
10-27-42	92.8±0.2	.41±.01	.35±.01	78	10	12	.24±.01	58	20	22	.23±.01	53	15	32
10-28-42	92.7±0.2	.44±.00	.40±.01	82	12	6	.26±.00	60	24	16	.22±.02	50	24	26
Mean and average deviation	.....	.....	.....	78±2	12±1	10±2	.....	60±3	24±2	16±4	.....	51±1	20±4	29±2

[1] Wisconsin Golden Acre variety.

in the ascorbic acid content of cabbage stored for six months. The figure in the present study, approximately nine per cent for three months, seems consistent with their higher figure for a longer period.

There was a significant difference in the amounts of ascorbic acid retained in cabbage cooked in different amounts of water; the figures for these experiments are given (Table 2). When the weight of water used was half the weight of cabbage, 78 ± 2 per cent of the ascorbic acid remained in the cooked cabbage; when twice as much water as cabbage was employed in cooking, 60 ± 3 per cent remained; and when four times as much water as cabbage was used, only 51 ± 1 per cent of the original ascorbic acid was retained. Approximately twice as much ascorbic acid was dissolved in the medium and large amounts of cooking waters as in the small amounts. The loss in ascorbic acid increased as the volume of water used in cooking was increased. The percentages of ascorbic acid retained by the drained, boiled cabbage are higher than those obtained by other workers, but the cooking conditions in this study favor greater retention. Wellington and Tressler (1938) boiled 360 grams of cabbage, cut in ½-inch strips, in 1,800 ml. of water for 10 minutes and found that 32 per cent of the original ascorbic acid remained in the drained cabbage. However, the proportion of water to cabbage used is higher than the four-to-one ratio used with the largest amount of water in the present study. Oser, Melnick, and Oser (1943) investigated the retention of vitamins and minerals in four vegetables when cooked by "new-improved" and "old-fashioned" methods. They reported losses of ascorbic acid of 14 per cent for peas and 33 per cent for broccoli when cooked by the "new-improved" method. Loss of approximately 22 per cent for cabbage cooked in a small amount of water in this study compares favorably with these figures.

The results obtained when boiling periods of different lengths were used (Table 3) indicate that when cabbage was boiled in twice its weight of water for 7-, 15-, and 25-minute periods, 55 ± 2, 52 ± 1, and 52 ± 2 per cent, respectively, of the original ascorbic acid remained. Under the conditions of this experiment, increasing the length of the cooking period approximately three and one-half times, did not significantly decrease the amount of ascorbic acid in the cooked cabbage. Gould, Tressler, and King (1936) boiled 360 grams of cabbage in 1,800 ml. of water for 12 minutes and found that the amount of ascorbic acid in the cabbage decreased, while the concentration in the cooking water increased during this period. In this experiment the concentrations of ascorbic acid in the cooking waters averaged 0.064, 0.121, and 0.188 milligram per ml., respectively, for the 7-, 15-, and 25-minute cooking periods, but the volumes of cooking water decreased with longer times of cooking. It would be interesting to know whether more ascorbic acid would have been lost with longer cooking periods if larger amounts of water had been used.

In cooking cabbage by different methods to the same stage of "doneness", slightly more ascorbic acid was retained when cabbage was boiled for seven minutes in 800 ml. of water in a covered pan then when it was boiled for eight and one-half minutes in an uncovered pan or when it was

average deviation	.....	.....	78±2	12±1	10±2	.....	60±3	24±2	16±4	.....	51±1	20±4	29±2

' Wisconsin Golden Acre variety.

TABLE 3

Effect of Variations in Time of Boiling on Ascorbic Acid Content of Cabbage[1]

Date	Raw cabbage		400 gm. cabbage in 800 ml. water, boiled for 7 min.				400 gm. cabbage in 800 ml. water, boiled for 15 min.				400 gm. cabbage in 800 ml. water, boiled for 25 min.			
	Moisture mean and average deviation	Ascorbic acid mean and average deviation	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost
	pct.	*mgm./gm.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*
10-30-42	93.2±0.3	.42±.01	.24±.02	58	25	17	.23±.01	53	32	15	.25±.02	55	28	17
11-3-42	93.0±0.1	.44±.01	.23±.02	53	24	23	.24±.00	53	32	15	.25±.02	53	28	19
11-4-42	93.1±0.2	.40±.02	.23±.01	56	24	20	.21±.01	52	31	17	.22±.01	51	28	21
11-5-42	93.2±0.2	.39±.01	.21±.00	54	20	26	.20±.01	51	31	18	.20±.01	50	28	22
Mean and average deviation	.....	.....	.....	55±2	23±2	22±3	.....	52±1	32±0	16±1	.....	52±2	28±0	20±2

[1] Wisconsin Golden Acre variety.

TABLE 4

Effect of Variations in Cooking Procedures on Ascorbic Acid Content of Cabbage[1] *Cooked to Same Degree of "Doneness"*

Date	Raw cabbage		400 gm. cabbage in 800 ml. water, boiled for 7 min., covered				400 gm. cabbage in 800 ml. water, boiled for 8.5 min., uncovered				400 gm. cabbage in 1,600 ml. water, boiled for 5.5 min., uncovered			
	Moisture mean and average deviation	Ascorbic acid mean and average deviation	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost	Ascorbic acid mean and average deviation	Retention	In cooking water	Lost
	pct.	*mgm./gm.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*	*mgm./gm.*	*pct.*	*pct.*	*pct.*
12-16-42	93.2±0.2	.40±.01	.23±.00	54	19	27	.19±.01	47	22	31	.21±.02	51	10	39
12-17-42	93.2±0.4	.36±.00	.21±.00	58	23	19	.20±.02	54	26	20	.18±.01	49	17	34
12-18-42	92.5±0.2	.42±.01	.22±.02	55	19	26	.21±.00	50	22	28	.20±.01	48	16	36
Mean and average deviation	.....	.....	.....	56±2	20±2	24±3	.....	50±2	23±2	26±4	.....	49±1	14±3	36±2

[1] Resistant Detroit variety.

boiled for five and one-half minutes in 1,600 ml. of water in an uncovered pan; the detailed figures are given (Table 4).

When 400-gram portions of cabbage were boiled in 800 ml. of water for seven minutes, held in refrigerator for 24 and 72 hours, and reheated, there were significant losses of ascorbic acid (Table 5). The cabbage tested immediately after cooking was found to retain 57±2 per cent of its ascorbic acid; the retention after holding the cooked cabbage for 24 hours and reheating was 29±3 per cent; and after holding similar cabbage for 72 hours and reheating, 24±4 per cent. The difference between the ascorbic acid contents of the two reheated cabbage samples is not significant. The cooking waters of the reheated cabbage contained between

TABLE 5

Ascorbic Acid Content of Cabbage[1] When Raw, Cooked, and Reheated After Storage

Date	Raw cabbage		Cooked cabbage		Cooked cabbage, stored 24 hr. and reheated		Cooked cabbage, stored 72 hr. and reheated	
	Moisture mean and average deviation	Ascorbic acid mean and average deviation	Ascorbic acid mean and average deviation	Retention	Ascorbic acid mean and average deviation	Retention	Ascorbic acid mean and average deviation	Retention
	pct.	*mgm./gm.*	*mgm./gm.*	*pct.*	*mgm./gm.*	*pct.*	*mgm./gm.*	*pct.*
11-6-42	93.2±0.1	.38±.02	.21±.02	56	.10±.00	24	.09±.01	21
11-10-42	93.4±0.1	.39±.01	.22±.01	54	.13±.01	28	.09±.00	20
11-11-42	93.0±0.1	.42±.01	.24±.01	60	.14±.00	28	.14±.01	28
11-27-42	93.3±0.1	.41±.02	.23±.00	57	.16±.01	35	.13±.01	29
Mean and average deviation	.....	.....	.....	57±2	.....	29±3	.....	24±4

[1] Wisconsin Golden Acre variety.

three and four per cent of the original ascorbic acid. Since ascorbic acid determinations were made only immediately after boiling and after holding and reheating, it is impossible to say whether the loss occurred during cooling, during the first 24 hours of storage, or when the cabbage was reheated. Gould and Tressler (1936) found that when cooked cabbage was stored in the refrigerator for 24 hours, approximately one-fourth of the ascorbic acid was lost and that in two days the loss averaged 50 per cent.

The effect on the ascorbic acid content of allowing shredded cabbage to stand in the air for one hour, and in water for one and three hours is presented (Table 6); the per cent retentions were, respectively, 97±2, 94±1, and 96±2.

Evidently the losses owing to these types of advance preparation are not large. Of two lots of cabbage purchased on the market, the results with one confirmed those given (Table 6), while the other lot showed greater losses. With the second lot purchased, shredded cabbage was allowed to stand one hour in air, one hour in water, and three hours in water: the percentages of ascorbic acid retained were 97, 97, and 98, respectively. These heads of cabbage were similar in size to those grown

by the Department of Horticulture and the outer leaves were green, while the heads of cabbage in the lot purchased first were twice as large and the outer leaves were creamy with a greenish tinge. When this cabbage was shredded and allowed to stand in water for four hours, it retained 87 per cent of the ascorbic acid, and 87 per cent after standing in water for 24 hours. The losses obtained with the small-headed, green cabbages allowed to stand in air are similar to those reported by Pyke (1942).

TABLE 6

Ascorbic Acid Content of Cabbage[1] When Raw, After Shredding and Standing in Air, and After Shredding and Standing in Water

Date	Raw cabbage		Cabbage shredded and allowed to stand 1 hour in air		Cabbage shredded and allowed to stand 1 hour in water		Cabbage shredded and allowed to stand 3 hours in water	
	Moisture mean and average deviation	Ascorbic acid mean and average deviation	Ascorbic acid mean and average deviation	Retention	Ascorbic acid mean and average deviation	Retention	Ascorbic acid mean and average deviation	Retention
	pct.	*mgm./gm.*	*mgm./gm.*	*pct.*	*mgm./gm.*	*pct.*	*mgm./gm.*	*pct.*
11-24-42	93.6±0.2	.41±.02	.41±.01	99	.34±.01	93	.36±.01	97
11-25-42	93.4±0.3	.42±.00	.42±.01	99	.36±.00	94	.35±.00	94
12-1-42	93.2±1.0	.40±.00	.38±.00	94	.34±.00	94	.34±.00	94
12-2-42	93.7±0.2	.38±.01	.37±.00	95	.34±.00	97	.34±.01	100
Mean and average deviation	.....	.....	.....	97±2	.....	94±1	.....	96±2

[1] Racine Market variety.

SUMMARY

The ascorbic acid content of cabbage did not decrease when freshly harvested heads were stored in closed containers at —0.5 to 4°C. (31 to 39°F.) for two months, or when the heads were kept in the refrigerator for a week. Losses of ascorbic acid occurred during the third month of storage and when cabbage was held at room temperature for three days.

When cabbage was boiled for seven minutes in the proportions of one part by weight of cabbage to one-half, two, and four parts of water, the per cent retention of ascorbic acid decreased as the volume of cooking water increased.

When cabbage was placed in twice its weight of boiling water and boiled for 7-, 15-, and 25-minute periods there was no significant difference in the ascorbic acid retention of the three cooked samples.

Cabbage was cooked to the same degree of "doneness" by three methods: boiling covered in twice its weight of water for seven minutes, boiling uncovered in twice its weight of water for eight and one-half minutes, and boiling uncovered in four times its weight of water for five and one-half minutes. Slightly more ascorbic acid was retained when cabbage was cooked by the first method, while no differences in ascorbic acid content were observed when cabbage was cooked by the second and third methods.

When cooked cabbage was held in the refrigerator for one and three days and then reheated, there were additional losses of approximately 28 and 33 per cent of the original ascorbic acid.

Losses in ascorbic acid content owing to advance preparation—shredding cabbage and allowing it to stand for one hour in air or for one and three hours in water—did not exceed 6 ± 1 per cent.

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RESPONSE OF LABORATORY ANIMALS TO CELLULOSE GLYCOLIC ACID AND ITS SODIUM AND ALUMINUM SALTS

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Cellulose glycolic acid is a hydrophilic colloid which is derived through the etherification of cellulose with chloroacetic acid. By suitable methods of preparation, a uniformly substituted product containing 0.4 to 0.8 carboxymethyl groups per anhydroglucose unit of the cellulose may be obtained. This type of cellulose glycolic acid is sold in the United States under the trade name of Collocel,[1] while the corresponding aluminum salt is available under the trade name Collocel A[1] and the water-soluble sodium salt as Collocel S.[1] Since these products have been found to be excellent emulsion stabilizers, thickeners, and binding agents, they can be used to advantage in food and pharmaceutical preparations. On this account it is necessary that information concerning their physiological activity be obtained.

Brown and Houghton (1941), in considering the sodium salt of carboxymethyl cellulose (sodium cellulose glycolate) containing 0.3 carboxymethyl radicals per anhydroglucose unit, state: "Tests on animals show that it is harmless physiologically and can therefore be used in edible preparations." Their test material was manufactured by the Imperial Chemical Industries, Limited, and sold under the trade name of "Cellofas WFZ." Werle (1941) has also shown that a sodium cellulose glycolate product, known commercially as "Z25," is harmless when fed to rats and dogs for a two-month period.

MATERIAL

The cellulose glycolic acid and aluminum cellulose glycolate used are white, dense, granular powders. Both of these products are insoluble in water and in all of the common organic solvents. They are readily dissolved, however, in dilute aqueous solutions of inorganic bases and some organic bases. Sodium cellulose glycolate in the form of a white, light, fluffy solid[2] was used in these experiments. It forms clear, colorless, aqueous solutions which are extremely viscous fluids even at three-per cent concentration and are gelatinous at five-per cent concentration; analytical data concerning the samples tested are given (Table 1).

DIETARY FEEDING

Control and Basic Diet: A modified Sherman diet devised by Hawk and Oser and published by Hawk and Bergeim (1931) was used as the

[1] Product of The Dow Chemical Company, Midland, Michigan.

[2] Sodium cellulose glycolate is also now available in the form of a dense, granular powder.

TABLE 1

Characteristics of Materials

Analysis	Cellulose glycolic acid	Sodium cellulose glycolate	Aluminum cellulose glycolate
Viscosity of 1% solution in centipoises	3.1[1]	23.0[2]	4.5[1]
	pct.	*pct.*	*pct.*
Water solubility	Insoluble	98.1	Insoluble
Ash	0.05	21.0	7.94
Chlorine (Parr Bomb)	0.092	0.042	0.86
Sulfate	Nil	Nil	0.60
Aluminum	Nil	Nil	3.87
Sodium	Nil	6.15	1.21
Nickel	Nil	Nil	0.017
Copper	0.0026	0.0095	0.0048
Carbon	41.92	36.98	37.08
Hydrogen	6.24	5.81	5.82

[1] Determined in aqueous caustic solution at pH of 12. [2] Determined in aqueous solution.

control diet throughout the experiment. This basic diet, designated as Diet I, was made up monthly and contained the following ingredients:

Whole wheat, freshly ground	55% by weight
Dried whole milk	25% by weight
Dried meat residue	12% by weight
Dried brewer's yeast	5% by weight
Calcium carbonate	1% by weight
Iodized table salt	2% by weight

This diet, as well as the experimental diets, was supplemented twice a week with approximately three grams of cabbage per rat.

Experimental Diets: Each of the experimental diets was made up by weight to contain five or 20 per cent of the test material and 95 or 80 per cent, respectively, of Diet I, the basic diet.

Experiment 1

Diet I — control
Diet II — contained 5% cellulose glycolic acid
Diet III — contained 5% sodium cellulose glycolate
Diet IV — contained 5% aluminum cellulose glycolate

Experiment 2

Diet I — control
Diet V — contained 20% cellulose glycolic acid
Diet VI — contained 20% sodium cellulose glycolate
Diet VII — contained 20% aluminum cellulose glycolate

Animals: All of the rats used in this work were the progeny of stock obtained from the Wistar Institute. At the age of 21 days all the animals were weaned and placed on Diet I. The rats were fed from stainless-steel hoppers which were weighed and refilled three times a week. Records of the food consumed were kept for each of the four groups in both experiments. Water was provided *ad libitum*.

All of the animals that were killed, as well as most of those that died during the experiment, were examined for gross pathological lesions. Representative animals in all groups were examined for histopathological changes.

Experiment 1: The first experiment involved the feeding of Diets I, II, III, and IV. For this experiment 100 young animals 22 to 32 days of age (average 25 days) were matched according to litter, sex, and weight. The sexes were caged separately and 10 males and 15 females were placed on each of the four diets. All animals were weighed twice weekly and individual weight records were kept throughout the experimental period of 201 to 250 days.

Experiment 2: The second experiment involved the feeding of Diets I, V, VI, and VII. For this experiment 40 practically mature females varying from 82 to 98 days of age were matched according to age and weight and 10 animals were placed upon each of the four diets. All animals were weighed twice weekly and individual weight records were kept throughout the experimental period of 63 days.

ORAL FEEDING

The acute oral toxicity was determined by administering the test materials to rats, rabbits, and guinea pigs by means of a stomach tube. Single doses of cellulose glycolic acid and its aluminum salt were fed as suspensions in olive oil and aqueous gum arabic while the sodium salt was fed as a three-per cent aqueous solution in three divided doses over a six-hour period. Control animals were treated in a comparable manner by administering similar volumes of the carriers employed. All animals except those that were autopsied for gross and histopathological studies were observed for a period of two weeks following feeding.

SKIN IRRITATION

The test materials were repeatedly bandaged to the shaven bellies of rabbits. Five observations and applications per week were made over a four-week period.

DISCUSSION OF RESULTS

DIETARY FEEDING

Experiment 1—Feeding of Diets Containing Five Per Cent of Test Materials: Throughout the experiment there was no detectable difference in the appearance of the control rats and those on the experimental diets. Pneumonia caused practically all of the deaths which occurred during the experiment, the incidence being the same in the control group as in the experimental groups.

Food consumption records indicate that during the early part of the experiment when the animals were small and growing rapidly they were consuming as much as six grams of the test material per kilogram of body weight per day. This daily dosage gradually decreased as the animals grew larger until the daily dosage was approximately two to three grams per kilogram of body weight for the mature animals.

Composite weight curves for the animals maintained on Diets I, II, III, and IV are presented (Fig. 1) and the organ weights as obtained at autopsy of the animals after 201 to 250 days on the experimental diets (Fig. 2). It is apparent that there were no significant differences between the average weight curves or between the organ weights of the rats from each experimental group. Furthermore, tissues from representative ani-

mals in each group (about 40 per cent of all animals) were studied histopathologically and in no instance was there evidence of pathological lesions. The liver, kidney, spleen, pancreas, adrenal, testis, and gastrointestinal tract were routinely examined.

Experiment 2 — Feeding of Diets Containing 20 Per Cent of Test Materials: Throughout the experiment there was no detectable difference in the gross appearance of the control rats and those on the experimental diets. Three deaths occurred during the course of the experiment and all were due to pneumonia. A softening of the feces was noted throughout the experiment in those animals maintained on Diet VI (20 per cent sodium cellulose glycolate) and also, but to a lesser extent, in those on Diet VII (20 per cent aluminum cellulose glycolate).

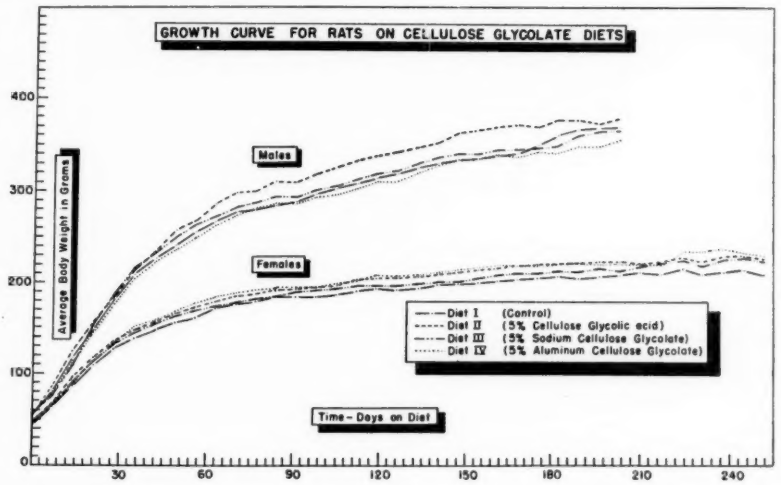

FIG. 1.

Composite weight curves for animals on Diets I, V, VI, and VII (Fig. 3) indicate that the animals on Diet V grew as well as did the controls on Diet I, whereas those on Diets VI and VII failed to make equivalent weight gains. The deviation from the control curve of the animals on Diet VI (sodium salt) was very slight and of questionable significance, while that of the animals on Diet VII (aluminum salt) was definite although not severe and was indicative of a possible ill effect.

Food consumption records (Table 2) indicate that each rat consumed approximately 11 to 13 grams of the diet per day or approximately 11 to 13 grams of the test material per kilogram of body weight per day. Such a dose is far larger than can be fed by stomach tube and constitutes a very severe test. Furthermore, if the quantity of food consumed is considered from a bulk standpoint it is apparent that those animals on Diet VI were eating almost one and one-half times as much as the control animals and yet were consuming only about 75 per cent as much of the nutrient food. This decrease in nutrient food intake (assuming no nutri-

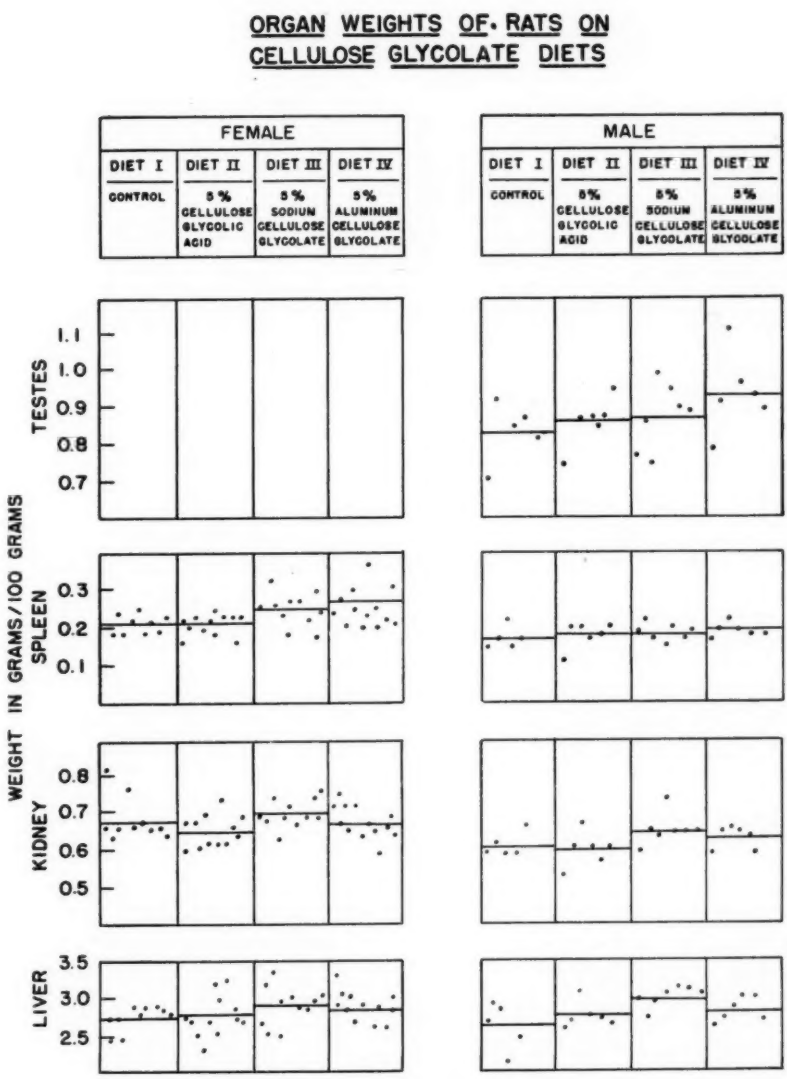
ORGAN WEIGHTS OF RATS ON CELLULOSE GLYCOLATE DIETS
FEMALE
MALE
DIET I
CONTROL
DIET II
5% CELLULOSE GLYCOLIC ACID
DIET III
5% SODIUM CELLULOSE GLYCOLATE
DIET IV
5% ALUMINUM CELLULOSE GLYCOLATE
WEIGHT IN GRAMS/100 GRAMS
TESTES
1.1
1.0
0.9
0.8
0.7
SPLEEN
0.3
0.2
0.1
KIDNEY
0.8
0.7
0.6
0.5
LIVER
3.5
3.0
2.5

FIG. 2.

ent value from the sodium cellulose glycolate) could easily account for the very slight growth curve depression apparent (Fig. 3). Animals on Diets V and VII were eating about the same volume of food as the controls and were obtaining about 95 per cent as much of the nutrient food.

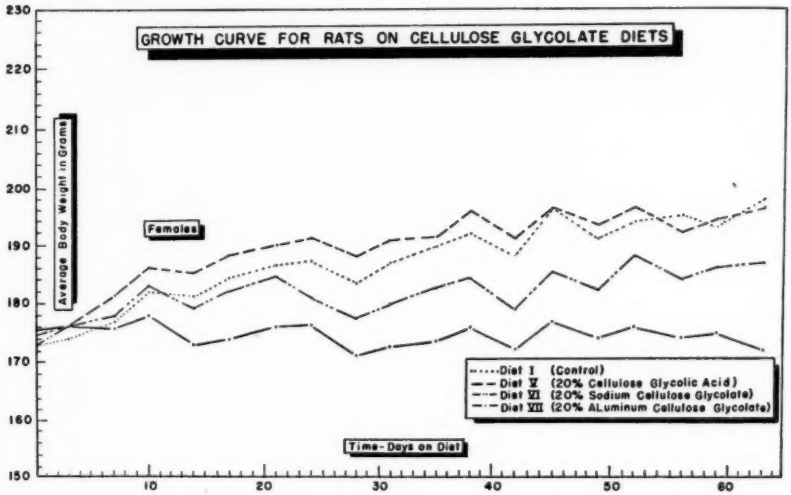

FIG. 3.

Gross pathological studies were made on all animals at autopsy. The gastrointestinal tract was carefully examined for any sign of irritation because of the poor pellet formation observed during the course of the experiment, but none was found. The intestinal contents were very bulky in all cases, there being a considerable quantity of the test material present. The intestinal contents of animals on Diet V were of normal consistency, while those on Diet VI were somewhat fluid and those on Diet VII were definitely soft.

TABLE 2

Food Consumption

Diet	Containing 20% by weight	Specific gravity	Average amount consumed per rat per day		Diet I obtained
			gm.	*c.c.*	*pct.*
I	Control	0.75	11.1	14.8	100
V	Cellulose glycolic acid	0.86	13.2	15.4	95
VI	Sodium cellulose glycolate	0.51	10.8	21.3	75
VII	Aluminum cellulose glycolate	0.86	13.0	15.1	94

Organ weights obtained at autopsy (Fig. 4) show that there was no significant deviation between any of the groups although a greater spread in spleen weights among the animals fed on Diet VII was noted.

Histopathological studies were conducted on all nine of the surviving animals on Diet VII and five animals each on Diets I, V, and VI. The liver, kidney, spleen, adrenal, and pancreas were routinely examined. The

only sign of pathological change was a slight congestion observed in the spleens from some of the animals on Diet VII.

ACUTE ORAL ADMINISTRATION

Rats, guinea pigs, and rabbits withstood single oral doses of cellulose glycolic acid (five grams per kilogram), sodium cellulose glycolate (three

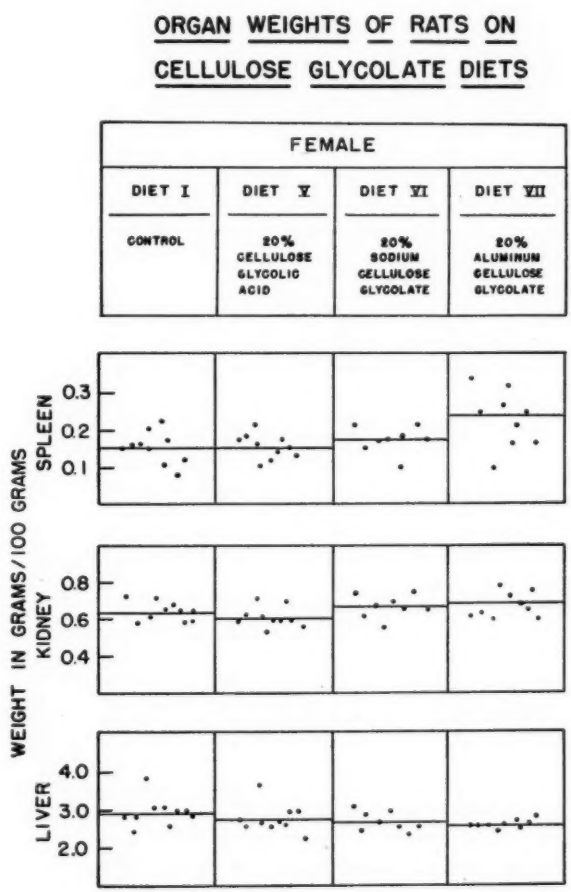

FIG. 4.

grams per kilogram in three divided doses), and aluminum cellulose glycolate (five grams per kilogram) without symptoms. These doses were the largest that could be fed by stomach tube because of the physical nature of the material. Gross and microscopic examination of tissues from the rats and guinea pigs taken at daily intervals after the forced feeding failed to reveal any pathological lesions except those which might be expected from the administration of large volumes of material.

SKIN IRRITATION

Cellulose glycolic acid, sodium cellulose glycolate, and aluminum cellulose glycolate were not irritating to the skin of rabbits.

SUMMARY

Young rats were maintained for a period of eight months on diets containing five per cent by weight of cellulose glycolic acid, sodium cellulose glycolate, or aluminum cellulose glycolate without producing any discernible ill effects as determined by growth curves, organ weights, gross observations, and histopathological examination. This is equivalent to the ingestion of two to three grams per kilogram of body weight per day over a period of eight months.

Practically mature female rats were maintained for two months on diets containing 20 per cent by weight of the test materials. This is equivalent to feeding 11 to 13 grams per kilogram of body weight per rat per day over a period of two months. The following observations were made:

(a) Cellulose glycolic acid produced no discernible ill effect as determined by growth curves, organ weights, gross observations, and histopathological examination.

(b) Aluminum cellulose glycolate produced a slight depressing effect upon the growth curve, a slight enlargement and congestion of the spleen, and a softening of the feces manifest by poor pellet formation. The general appearance of the rats was entirely normal.

(c) Sodium cellulose glycolate produced no effect upon organ weights nor any histopathological changes. A very slight growth-curve depression of questionable significance can well be explained by the decrease in nutrient food intake owing to the bulkiness of Diet VI. The general appearance of the animals was normal except that their feces were somewhat soft throughout the experimental period.

The acute oral toxicities of cellulose glycolic acid and its sodium and aluminum salts are low for rats, guinea pigs, and rabbits. Amounts as large as can be fed cause no ill effects.

Cellulose glycolic acid and its sodium and aluminum salts are not irritating to the skin of rabbits.

CONCLUSION

The results of animal-feeding experiments on cellulose glycolic acid, sodium cellulose glycolate, and aluminum cellulose glycolate show no evidence why these materials should not be incorporated into the food or pharmaceutical preparations designed for human consumption.

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A RAPID TEST FOR DETECTION OF SPOILAGE IN SEA FISH

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(Received for publication, September 20, 1943)

A satisfactory test for the detection of spoilage in fish must meet certain definite requirements. It should provide an accurate measure of the degree of spoilage and should be based on the most characteristic change occurring in the product as spoilage progresses. It should permit a prediction of the keeping time if the test indicates that the fish is in the incipient stage of spoilage. Finally, the test should be rapid and simple to perform. From the practical standpoint the latter requirement probably takes on as great significance as any other because the great variation in quality of any given lot of fish or fillets necessitates the testing of large numbers of samples if a truly representative analysis is to be obtained.

The trimethylamine value of Beatty and Gibbons (1937) and a tyrosine value modified from Bradley and Bailey (1940) have both yielded useful results in this laboratory. Recently Wood, Sigurdsson, and Dyer (1942) have shown that these values can be made more satisfactory through the use of a surface sampling procedure. Such a method of sampling permitted the detection of spoilage earlier than when composite samples were used. This was shown to be due to the fact that the bacteria responsible for the spoilage changes are normally present and most active on the surface of the fish. The more rapid appearance and accumulation of trimethylamine and tyrosine at the surface are manifestations of this higher bacterial population in the narrow surface zone.

It has been found that the above tests are too time consuming for routine use with large numbers of samples. The increase in trimethylamine at the surface is reflected in a definite rise in pH as spoilage progresses. On several occasions the pH of fish muscle has been proposed as an index of spoilage—Van Deurs and Hoff-Jorgensen (1936), Charnley and Goard (1942)—although in most cases such tests have been found to be of doubtful value—Tarr and Sunderland (1941). In so far as the writers are aware composite sampling methods have been used in the previous work on pH as a measure of spoilage. It is the purpose of the present paper to show that the pH of fish muscle is a reliable index of quality providing that the surface hydrogen-ion concentration is considered and the test is applied to marine fish in which trimethylamine oxide is a muscle constituent.

EXPERIMENTAL PROCEDURE

The trimethylamine determinations were made by the method of Beatty and Gibbons (1937). For the tyrosine values a modification of the Bradley and Bailey (1940) procedure, as described by Wood, Sigurdsson, and Dyer (1942), was followed. The Beckman pH meter (laboratory model) with glass electrode was used for the pH measurement. The surface sam-

ples for trimethylamine and tyrosine determinations were obtained by cutting off the surface layer of tissue to a depth of three millimeters. At the outset the pH values were obtained on samples diluted with an equivalent weight of water. Subsequent studies demonstrated that the pH values could be obtained with equal accuracy by touching the electrodes directly to the surface of the fish or fillet. Results obtained by both methods of pH determination on a cod fillet stored at 10°C.(50°F.) are given (Table 1).

TABLE 1

Method of pH Determination

Time of storage	Direct surface value	Surface sample removed and dispersed in water
hr.		
0	6.50	6.50
24	6.60	6.55
48	6.70	6.70
72	7.30	7.30
96	7.70	7.70
120	7.90	8.00
144	7.95	8.00

The surface pH obtained by direct electrode contact is the same, within experimental error, as that of the three-mm. surface sample dispersed in an equivalent weight of water.

SURFACE PH CHANGE IN STORED COD FILLETS

A cod fish was brought alive to the laboratory, killed, and immediately filleted. The change in hydrogen-ion concentration was followed on storage at room temperature of 20°C.(68°F.) until the fillet had become spoiled; the values obtained are given (Table 2).

The initial increase in hydrogen-ion concentration has been noted in the case of all strictly fresh fish examined. This result is in agreement

TABLE 2

Change in pH of Spoiling Cod Muscle

Time	Surface pH	Organoleptic examination
hr.		
0	6.50	Fresh
2	6.45	Fresh
4	6.45	Fresh
6	6.45	Fresh
8	6.40	Fresh
10	6.35	Fresh
20	6.55	Fresh
22	6.70	Fresh
24	6.90	Spoiling
26	7.10	Spoiling
28	7.35	Spoiled
36	7.60	Spoiled
48	7.95	Badly spoiled

with the findings of Benson (1928). In commercial fish or fillets this initial fall in the surface pH may not be noted if the muscle has passed through this primary change prior to examination. The reasons for this pH change are not entirely clear but may probably be attributed to autolytic changes occurring in the development of rigor, according to Ritchie (1925).

The relation of the surface pH to the pH of a composite sample of the same fish or fillet was next considered. Values obtained from commercial haddock fillets stored at 5° C. (41° F.) for various times are presented (Table 3); the pH of the interior of the fillets, obtained by direct contact of the electrodes with the cut flesh, is also given.

TABLE 3

Relation of Surface to Interior and Composite pH Values

Storage time	Surface	Interior	Composite
days			
0	6.40	6.25	6.35
2	6.60	6.35	6.55
3	7.00	6.35	6.60
4	7.20	6.40	6.65
5	7.50	6.50	7.00
6	8.00	6.90	7.40

These results demonstrate quite forcibly the much earlier rise of pH in the surface layer as compared with the interior or composite samples. The interior, at the beginning of the experiment, had a pH of 6.25 as compared with a surface pH of 6.4. After five days of storage the surface had reached pH 7.5 while the interior had reached pH 6.5. In the same period the pH of the composite sample increased from 6.35 to 7.0. This change in pH of the composite sample is of sufficient magnitude to indicate spoilage but the change is not as great nor does it occur as rapidly as on the surface. For instance, the surface had reached pH 7.0 after three days while the composite sample required five days to reach this level. This general relationship has been found to hold in all cases examined.

From the point of view of a commercial test, the surface sampling procedure is much more satisfactory since the samples are not destroyed; the reading is taken almost instantaneously, so that spoiled or nearly spoiled samples may be rejected or rerouted for immediate consumption; and an indication is given of incipient spoilage.

In this case (Table 3) the surface pH does not show the initial fall shown in the data in Table 2. This was expected since the fillets used for the experiment had been stored commercially for five days at 0°C. (32°F.) before being brought to the laboratory, with the result that the surface pH had dropped to its lowest value and then was beginning to increase. Such a condition can, in general, be detected through a parallel determination of the surface and interior pH. If both the surface and interior values show pH levels of 6.5 to 6.8, the fish is probably quite fresh. Later both values decrease together to about the range 6.25 to 6.4,

three to five days at 0°C.(32°F.). Longer storage or incipient spoilage is shown by an increase in the surface value to 6.9 or 7.5 with a very slight rise in the interior. Later, in spoiled fish the surface may reach pH 8 or higher, and the interior rapidly approaches this level.

RELATIONSHIP OF PH TO TRIMETHYLAMINE AND TYROSINE VALUES

A comparison of the surface trimethylamine, the surface tyrosine, and the surface pH values with composite values on the same cod fillets (Table 4) demonstrates that there is satisfactory agreement among all

TABLE 4

Surface and Composite pH, Trimethylamine, and Tyrosine Values of Cod Fillets Stored at 5°C.(41°F.)

Storage time	pH value		Trimethylamine value[1]		Tyrosine value[1]		Organoleptic examination
	Surface	Composite	Surface	Composite	Surface	Composite	
days							
2	6.70	6.50	10.0	5.5	16.0	10.6	Fresh
3	7.30	6.90	46.0	18.0	18.5	11.4	Spoiling
4	7.70	7.30	66.0	40.0	28.0	16.0	Spoiled
5	7.90	7.40	78.0	47.5	30.0	24.6	Badly spoiled

[1]Expressed in milligrams per 100 grams of muscle tissue.

three tests. It is equally apparent that the surface sampling greatly accentuates the changes under examination with all three indices. The progressive rapid increase of the relatively strong base, trimethylamine ($pK_b = 4.13$), provides an explanation for the corresponding pH changes. It has been shown by Wood, Sigurdsson, and Dyer (1942) that the interior pH rises through diffusion of the trimethylamine from the surface.

DISCUSSION

The above data show conclusively that in a perishable food product, such as fish or fish fillets, where spoilage occurs almost entirely through bacterial action at the surface, the measurement of the end products of such bacterial action in the surface layer of the tissue provides the most satisfactory index of spoilage. In sea fish, where the strong base trimethylamine is almost invariably produced by the heterogeneous flora present on the surface, the simple measurement of surface pH by direct contact affords an extremely rapid and convenient method for the routine or laboratory determination of the spoilage index. The surface method may also have application to other food products where oxidative or other changes producing measurable products constitute in part at least the spoilage mechanism.

The pH test has been applied over a period of two years on cod, haddock, and flounder with satisfactory results. It can be used with equal ease on round fish or fillets. The versatility of the direct reading pH meter with glass electrode permits of simple and easy operation. For commercial use, the conventional scale of the galvanometer or pH calibrated potentiometer could be replaced with one indicating directly the state of freshness of the fish as shown in the diagrammatic sketch (Fig. 1).

The exact pH level at which a fish may be considered as spoiled will depend to a considerable extent upon the taste of the consumer, there being a considerable range in different parts of the country in this respect. Whether a fish or fillet with a surface pH of 6.8, 6.9, or 7.0 will be considered spoiled can thus only be determined by commercial practice. Over a period of time the results obtained under practical conditions will point the way to the level that may be assigned to fish in various stages of spoilage.

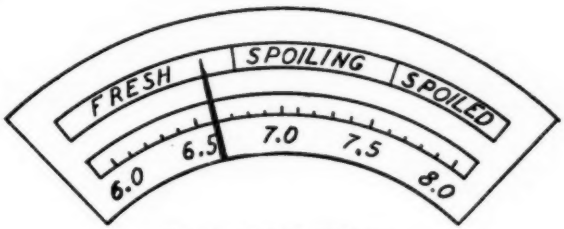

FIG. 1. Scale of freshness.

SUMMARY

A satisfactory routine practical method for the determination of the index of spoilage for several sea fish has been developed. The pH at the surface of the fillet or fish is measured by placing a glass electrode of a Beckman pH meter in contact with the moist surface of the tissue. It has been found that fresh fish have a pH range of 6.2 to 6.8; pH levels above 6.8 are indicative of spoilage; and the higher the pH above 6.8 the more extensive have been the spoilage changes.

This method is simple, rapid, and adapted for use as a commercial test. Correlation with other spoilage tests is satisfactory. A much better indication of conditions of incipient spoilage is afforded than that obtained by currently used methods. The standard scale of the direct reading pH meter may be replaced by one showing the degree of freshness of the fisheries product.

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SOME ANTIOXIDANT PROPERTIES OF D-ISO ASCORBIC ACID AND ITS SODIUM SALT[1]

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The purpose of this investigation was to study the properties and reactions of d-iso ascorbic acid and its sodium salt in order to evaluate them as antioxidants.

L-ascorbic acid has been suggested and used in preserved foods to prevent or retard oxidation with its resultant discoloration and off-flavor effects. From the economic standpoint it would be preferable to employ d-iso ascorbic acid for this purpose if it were equally effective. The close relationship of these two compounds is shown by the fact that the dye-titration method of determining l-ascorbic acid, based on the oxidation of the acid by the dye sodium 2,6-dichlorobenzenoneindophenol, cannot differentiate between the two acids.

The sodium salt of d-iso ascorbic acid is an intermediate product in the manufacture of d-iso ascorbic acid. From its chemical structure it would appear to be a practical antioxidant, although it would be considerably less effective than d-iso ascorbic acid on a molal basis.

REVIEW OF LITERATURE

Ascorbic Acids as Antioxidants: Gray and Stone (1939a) used l-ascorbic and d-gluco ascorbic acids as a means of preventing darkening and flavor changes in beer. Reductone and dihydroxy maleic acids were also used by Gray and Stone (1939b) in addition to the above ascorbic acids to control rancidity in oil emulsions, such as mayonnaise and salad dressings. Reductone and dihydroxy acid both contain the dienol group which gives the ascorbic acids their reducing properties.

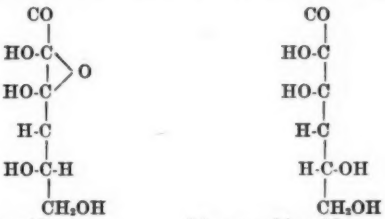
Fellers, Esselen, Fitzpatrick, Moore, and Powers (1942) made use of l-ascorbic, d-iso ascorbic, and d-gluco ascorbic acids as antioxidants in a variety of glass- and tin-packed foods naturally low in vitamin C. They found that the addition of small amounts of ascorbic acid was effective in controlling discoloration and undesirable flavor changes.

Moore (1942) added l-ascorbic, d-iso ascorbic, and d-gluco ascorbic acids to bottled orange juice to see if they would have any effect upon the quality of the juice after storage. He found that the development of off-flavors was delayed by the addition of any one of the ascorbic acids mentioned but that darkening of the juice was not prevented. Moore, Esselen, and Fellers (1942) concluded that oxygen and ascorbic acid are the principal factors involved in the darkening of packaged orange juice.

[1] Contribution No. 486, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

Greenbank (1940) reported that oxidized flavor develops in milk when its oxidation-reduction potential rises. The addition of l-ascorbic acid to milk prevented the development of oxidized flavor.

Properties and Reactions of D-iso Ascorbic Acid: D-iso ascorbic acid differs from l-ascorbic acid only in the relative position of the hydrogen and hydroxyl groups in the fifth carbon atom of the molecule. The structural formulae of these two ascorbic acids are presented below.



l-ascorbic acid d-iso ascorbic acid

Bachstez and Cavallini (1934) described a qualitative test for d-iso ascorbic acid based on a color reaction between the acid and uranyl acetate in a faintly alkaline solution. A light brown color develops in the solution, and the subsequent addition of more alkali intensifies the color. L-ascorbic acid also gives the brown color reaction but the addition of alkali discharges the color and brings down an amorphous precipitate of sodium diuranate.

Ohle, Erlbach, and Carls (1934) found the antiscorbutic activity of d-iso ascorbic acid to be one-fortieth that of l-ascorbic acid. Zilva (1935) studied the antiscorbutic activity and body retention of a number of ascorbic acids in guinea pigs. He reported the activity of d-iso ascorbic acid to be one-twentieth that of l-ascorbic acid.

Maurer and Scheidt (1934) studied the behavior of d-iso ascorbic and l-ascorbic acids towards ferrous salts. In the presence of oxygen and on the addition of ferrous salts the ascorbic acids developed a deep violet color. The color was considered to be due to the formation of a complex at the dienol group. When oxygen was excluded from the reaction the color did not develop.

D-iso ascorbic acid can be used as a developing agent in photographic work because of its reducing ability. Pavolini (1935) found it to be an excellent developer for overexposed films because it acted more slowly than the developers in common use. It gave a fine grain to the finished prints and prevented their fogging.

According to Bachstez and Cavallini (1937) the dissociation constants of d-iso ascorbic acid and l-ascorbic acid were 4.18 and 4.26, respectively, as determined by the method of conductivity in aqueous solutions. The acidity of the ascorbic acids was not due to a carboxyl group as might be assumed. Methylation studies on d-iso ascorbic acid by Hawkins, Hirst, and Jones (1939) indicated that the acidity of the molecule was due to the hydroxyl group on the third carbon atom of the molecule. The lactone ring present forms a carboxyl group when the oxidation of the acid proceeds rapidly, but it does not break down by treatment with strong alkali

alone. This feature of the ascorbic acids is unique because lactone rings are usually very unstable to alkalis. This stability was attributed by Hirst (1939) to the protective action of the ionizable enolic groups present in the molecule.

Johnson and Zilva (1937) studied the rate of enzymic oxidation of the ascorbic acids, including d-iso ascorbic acid, in relation to their stereochemical structure. The ascorbic acids having their lactone ring to the right were found to oxidize at a faster rate than their antiomorphs.

Bamdas, Lavrov, Rodinov, and Yarusova (1938) reported that sodium d-iso ascorbate had one five-hundredth the antiscorbutic activity of l-ascorbic acid.

EXPERIMENTAL PROCEDURE

Antiscorbutic Activity of D-iso Ascorbic Acid: In the literature it has been reported that d-iso ascorbic acid exhibits but little antiscorbutic activity; however, the data as reported are not in agreement.

As a preliminary step in this investigation the antiscorbutic activity of d-iso ascorbic acid was determined by the 25-day weight-response, bioassay method as described by Dunker, Fellers, and Esselen (1942). It was found that 10 mg. of d-iso ascorbic acid were approximately equivalent to 0.5 mg. of l-ascorbic acid (vitamin C) in antiscorbutic activity. On this basis d-iso ascorbic acid is apparently one-twentieth as active as l-ascorbic acid. These results are in agreement with those reported by Zilva (1935).

Relative Rates of Oxidation of L-ascorbic Acid, D-iso Ascorbic Acid, and Sodium D-iso Ascorbate: Solutions of l-ascorbic acid, d-iso ascorbic acid, and sodium d-iso ascorbate were made up to a concentration of 0.4 mg. per ml. in distilled water and were buffered to a pH value of 4 with secondary phosphate and citric acid. The solutions were put up in pint bottles at room temperature with head spaces of 15, 25, and 50 c.c., respectively, and sealed with crown caps having glazed paper "spots" over the cork gaskets. The bottles were then pasteurized at 75°C.(167°F.) for 30 minutes. The bottles were evenly divided into two groups, one group being stored in the dark at 60°C.(140°F.) and the other in the dark at 26°C.(78.8°F.).

At regular intervals samples of each of the ascorbic acid solutions were titrated with sodium 2,6-dichlorobenzenoneindophenol dye. The dye was standardized with pure l-ascorbic acid each day it was used. By this means the course of the oxidation in the bottled solutions could be followed. (Table 1).

When stored at 26°C. the differences in the rates of oxidation of l-ascorbic acid, d-iso ascorbic acid, and sodium d-iso ascorbate were small but, nevertheless, discernible. D-iso ascorbic acid oxidized more rapidly and sodium d-iso ascorbate more slowly than l-ascorbic acid as indicated by the changes in dye value.

At 60°C. there was a marked difference in the rates of oxidation of l-ascorbic acid and d-iso ascorbic acid. D-iso ascorbic acid was oxidized considerably faster than l-ascorbic acid. Sodium d-iso ascorbate oxidized at about the same rate as the l-ascorbic acid.

The rate of oxidation of each ascorbic acid increased markedly with the volume of the head space in the bottles. This illustrates the importance of a small head space in preventing or retarding oxidation.

Color Development by Solutions of L-ascorbic Acid, D-iso Ascorbic Acid, and Sodium D-iso Ascorbate During Storage: During some preliminary studies solutions of l-ascorbic and d-iso ascorbic acid developed a light yellow-brown color on standing for a long period of time. If ascorbic acids are used as antioxidants it is important to know just how much color is developed and what factors favor its development.

TABLE 1

Rate of Oxidation of Bottled Ascorbic Acid Solutions During Storage as Indicated by 2,6-dichlorobenzenoneindophenol Dye Titration

Storage time	L-ascorbic acid			D-iso ascorbic acid			Sodium d-iso ascorbate		
	Head space volume								
	15c.c.	25c.c.	50c.c.	15c.c.	25c.c.	50c.c.	15c.c.	25c.c.	50c.c.
	STORED AT 26°C.								
days	*mg. per ml.*			*mg. per ml.*			*mg. per ml.*		
0	.40	.40	.40	.40	.40	.40	.40	.40	.40
3	.30	.25	.19	.30	.28	.19	.33	.28	.22
6	.28	.24	.19	.28	.25	.13	.33	.28	.21
15	.28	.23	.12	.28	.24	.11	.32	.28	.21
29	.28	.23	.11	.27	.22	.10	.32	.26	.16
43	.28	.23	.10	.27	.22	.086	.32	.26	.15
64	.27	.22	.094	.25	.22	.086	.31	.26	.15
85	.26	.21	.092	.24	.20	.080	.30	.24	.12
235	.23	.19	.080	.20	.16	.057	.28	.23	.11
	STORED AT 60°C.								
0	.40	.40	.40	.40	.40	.40	.40	.40	.40
3	.29	.24	.14	.28	.26	.14	.31	.26	.18
6	.26	.21	.11	.24	.21	.094	.31	.25	.16
15	.26	.19	.09	.20	.18	.052	.28	.22	.10
29	.23	.17	.076	.16	.15	.049	.22	.18	.073
43	.21	.16	.065	.11	.11	.026	.21	.14	.057
64	.17	.13	.057	.062	.05	.017	.17	.12	.049
85	.14	.13	.043	.045	.04	.010	.13	.076	.040
235	.07	.063	.027	.040	.03	.006	.078	.039	.018

To determine the amount of color developed, quantitatively, the color in the bottled solutions of ascorbic acids being tested for relative rates of oxidation was determined by the use of an Evelyn photoelectric colorimeter. For this determination filter No. 400 was used.

The amount of color formed by the decomposition of the ascorbic acids was proportional to the amount of oxygen available in the head space and to the temperature at which the bottled solutions were stored (Table 2). L-ascorbic acid developed a more intense color than did d-iso ascorbic acid or its sodium salt. From the standpoint of discoloration it would appear that d-iso ascorbic is preferable to l-ascorbic acid as an antioxidant.

D-iso Ascorbic Acid and Its Sodium Salt as Antioxidants for L-ascorbic Acid: On the basis of the above experiments it was apparent that d-iso ascorbic acid oxidizes more rapidly than does l-ascorbic acid. With this

in mind, experiments were set up to determine whether or not d-iso ascorbic acid and its sodium salt would function as antioxidants for l-ascorbic acid in bottled solutions.

A solution of l-ascorbic acid, one milligram per milliliter in concentration, was made up and buffered to a pH value of 4. A similar solution was made up to which was added, in addition to the l-ascorbic acid, an equal amount of d-iso ascorbic acid. These solutions were put into pint

TABLE 2

Rate of Darkening of Bottled Ascorbic Acid Solutions During Storage

Storage time	L-ascorbic acid			D-iso ascorbic acid			Sodium d-iso ascorbate		
	Head space volume								
	15c.c.	25c.c.	50c.c.	15c.c.	25c.c.	50c.c.	15c.c.	25c.c.	50c.c.
	STORED AT 26°C.								
days									
0	100.0[1]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
3	98.0	95.0	94.0	97.0	99.0	99.0	100.0	99.0	97.0
6	97.6	95.0	93.0	97.0	96.0	93.0	100.0	98.0	97.0
15	94.0	93.5	88.0	97.0	96.0	91.0	98.0	97.5	96.0
29	94.0	93.0	83.0	97.0	95.5	89.0	97.0	97.0	93.0
43	94.0	93.0	80.0	96.0	93.5	89.0	96.5	96.5	91.0
64	93.0	81.0	77.0	93.0	92.0	86.0	96.5	96.0	90.0
85	91.5	88.5	75.5	93.0	91.5	86.0	96.5	95.5	88.0
235	86.0	82.0	65.0	93.0	89.0	82.0	93.0	91.0	86.0
	STORED AT 60°C.								
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
3	96.0	93.0	87.0	95.5	96.0	96.0	99.0	98.0	97.0
6	94.5	91.5	84.0	95.0	95.0	90.0	98.0	98.0	96.5
15	92.0	88.0	78.0	94.5	93.5	88.0	95.5	94.5	90.5
29	88.0	85.0	74.0	93.0	92.0	85.0	95.0	94.5	88.0
43	86.0	81.5	71.0	92.0	89.0	84.0	94.5	93.0	87.0
64	81.5	77.0	68.0	86.5	88.0	79.0	94.0	90.0	86.0
85	78.0	72.5	64.0	86.0	86.0	77.0	94.0	90.0	86.0
235	63.0	63.0	51.0	80.0	80.0	69.0	88.0	83.0	79.0

[1] Given in percentage of light transmission or light at 4,000 Å wave length. A high light transmission indicates a light-colored or clear solution; a low light transmission, a darkened or browned solution.

bottles and a head space of 25 c.c. was left at the top of each bottle. After being pasteurized for 30 minutes at 75°C., 30 bottles of each solution were stored at a temperature of 60°C. for 25 days. They were then removed from the incubator at 60°C. and put into a refrigerator at 5°C. where they were held until used. Previous experiments had shown that oxidation was rapid for the first 21 days and then slowed down as the amount of oxygen present decreased. This indicated that a 25-day oxidation period was sufficient to demonstrate whether or not d-iso ascorbic acid would be oxidized in preference to l-ascorbic acid in a mixture of the two.

A guinea-pig bioassay was used to determine the amount of biologically active l-ascorbic acid present in the mixtures. The assays were made according to the 25-day, weight-response method described by Dunker, Fellers, and Esselen (1942). The control animals were fed at levels of

0.2, 0.4, 0.6, and 0.8 mg. of l-ascorbic acid daily from the l-ascorbic solution as determined by titration with the dye sodium 2,6-dichlorobenzenoneindophenol. The test animals were fed at levels of 0.4, 0.6, and 0.8 mg. of mixed ascorbic acids from the mixed solution as determined by the dye titration. The supplements were fed with a one-ml. pipette graduated in hundredths of a milliliter. A fresh bottle of each solution was used each day in feeding the animals. Five guinea pigs were used to each level and each pig was kept in a separate cage; the weight responses of the animals to each level are shown (Table 3).

TABLE 3

Weight Responses of Guinea Pigs to Various Levels of Oxidized Mixtures of L-ascorbic and D-iso Ascorbic Acids

Solution	Original value dye	Dye value after storage (at time of feeding)	Level fed daily		Average weight gain
			Volume	Ascorbic acid [1]	
	mg./ml.	*mg./ml.*	*ml.*	*mg.*	*gm.*
1. L-ascorbic acid, 1.0 mg. per ml.	1.0	0.62	0.32	.2	—27.0
1. L-ascorbic acid, 1.0 mg. per ml.	1.0	0.62	0.64	.4	1.7
1. L-ascorbic acid, 1.0 mg. per ml.	1.0	0.62	0.97	.6	65.0
1. L-ascorbic acid, 1.0 mg. per ml.	1.0	0.62	1.29	.8	110.0
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml.	2.0	1.07	0.37	.4	25.0
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml.	2.0	1.07	0.56	.6	70.0
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml.	2.0	1.07	0.75	.8	108.0

[1] Based on dye value of ascorbic acid solution.

The animals on the 0.6- and 0.8-mg. levels of l-ascorbic acid gained almost exactly the same weight as those on similar levels of the mixture of l-ascorbic and d-iso ascorbic acids in the 25 days of the assay. This indicated that the antiscorbutic power of the two solutions was the same. The dye-titration value for the mixture would then be almost an exact index of the l-ascorbic acid present in the mixture. It may be assumed that the d-iso ascorbic acid present in the solution has been preferentially oxidized leaving the l-ascorbic acid untouched.

A second experiment was run to check the results obtained. In addition to the mixture of the two acids in equal concentration, a second mixture was made up containing one mg. l-ascorbic acid and 0.2 mg. d-iso ascorbic acid per milliliter. A third mixture was made up containing one mg. per ml. each of l-ascorbic acid and sodium d-iso ascorbate.

As in the first experiment the solutions were allowed to oxidize three weeks. The dye values were the measure of how much of each mixture should be fed the guinea pigs. The 0.2-mg. level was omitted because the weight response on this level did not give any significant data. The results of this second experiment are given (Table 4).

The weight responses of the guinea pigs fed Solution 2 were slightly less than those on the l-ascorbic control. In view of the fact that the mixture still had a dye value of 1.2 mg. per ml. when the oxidation was

stopped by refrigeration, this lower weight response was to be expected. There was only one mg. per ml. of l-ascorbic acid in the mixture and consequently the oxidized mixture with a dye value of 1.2 mg. per ml. could not protect as well as pure l-ascorbic acid when fed in equivalent amounts based on dye value.

The guinea pigs fed Solution 3 had weight responses almost the same as those on l-ascorbic acid. It is concluded, therefore, that the d-iso ascorbic acid present was completely oxidized leaving only l-ascorbic acid.

TABLE 4

Weight Responses of Guinea Pigs to Various Levels of Oxidized Mixtures of Ascorbic Acids

Solution	Original dye value	Dye value after storage (at time of feeding)	Level fed daily		Average weight gain
			Volume	Ascorbic acid [1]	
	mg./ml.	*mg./ml.*	*ml.*	*mg.*	*gm.*
1. L-ascorbic acid, 1.0 mg. per ml	1.0	0.58	0.69	.4	5
1. L-ascorbic acid, 1.0 mg. per ml	1.0	0.58	1.03	.6	80
1. L-ascorbic acid, 1.0 mg. per ml	1.0	0.58	1.38	.8	130
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml	2.0	1.22	0.32	.4	—3
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml	2.0	1.22	0.49	.6	75
2. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. d-iso ascorbic acid per ml	2.0	1.22	0.66	.8	117
3. Mixture of 1.0 mg. l-ascorbic acid plus 0.2 mg. d-iso ascorbic acid per ml	1.2	0.74	0.54	.4	2
3. Mixture of 1.0 mg. l-ascorbic acid plus 0.2 mg. d-iso ascorbic acid per ml	1.2	0.74	0.81	.6	85
3. Mixture of 1.0 mg. l-ascorbic acid plus 0.2 mg. d-iso ascorbic acid per ml	1.2	0.74	1.08	.8	129
4. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. sodium d-iso ascorbate per ml	1.7	1.08	0.37	.4	....[2]
4. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. sodium d-iso ascorbate per ml	1.7	1.08	0.56	.6	—4
4. Mixture of 1.0 mg. l-ascorbic acid plus 1.0 mg. sodium d-iso ascorbate per ml	1.7	1.08	0.74	.8	5

[1] Based on dye value of ascorbic acid solution. [2] All animals died of scurvy before test period was concluded.

Solution 4 containing l-ascorbic acid and sodium d-iso ascorbate in equal concentration did not give good weight responses. The guinea pigs on the 0.4-mg. level died of scurvy before the assay was completed. The animals on 0.6 and 0.8 mg. daily had poor weight responses indicating that sodium d-iso ascorbate did not protect the l-ascorbic acid in the mixture.

It is recognized that in the above bioassays any d-iso ascorbic acid present would contribute in a small way to the response of the animals. However, at the levels fed any such antiscorbutic effect would be insignificant and well within the experimental error.

SUMMARY

A study was made of the properties and reactions of d-iso ascorbic acid and its sodium salt, sodium d-iso ascorbate, that would affect their use as antioxidants.

The antiscorbutic activity of d-iso ascorbic acid was found to be one-twentieth that of l-ascorbic acid, vitamin C.

D-iso ascorbic acid oxidized more rapidly than l-ascorbic acid or sodium d-iso ascorbate during storage in a buffered aqueous solution.

Solutions of d-iso ascorbic acid, l-ascorbic acid, and sodium d-iso ascorbate developed an increasingly darker yellow-brown color as their oxidation progressed. The color developed to a lesser extent with d-iso ascorbic acid than with l-ascorbic acid and least with sodium d-iso ascorbate.

The factors influencing the development of color in the ascorbic acid solutions were found to be the amount of oxygen present, the temperature of storage, and the storage time.

D-iso ascorbic acid was found to protect the l-ascorbic acid from oxidation while the sodium d-iso ascorbate did not. The d-iso ascorbic acid was preferentially oxidized, in bottled aqueous solution, leaving the l-ascorbic acid unoxidized. It would therefore appear that d-iso ascorbic acid is an excellent antioxidant for l-ascorbic acid, vitamin C.

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RIBOFLAVIN REQUIREMENTS OF CERTAIN LACTIC ACID BACTERIA[1]

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Recent developments in the field of B-complex vitamin research have shown the value of certain bacteria as assay organisms. Several lactic acid types have been found to require one or more of the B vitamins to produce normal growth, the amount of acid produced in the presence of a certain number of the B complex being, within certain limits, in proportion to the quantity of the limiting vitamin present.

Orla-Jensen, Otte, and Snog-Kjaer (1936) stated that riboflavin is necessary for the normal development of certain lactic acid bacteria, such as *Thermobacterium lactis, Thermobacterium bulgaricum, Thermobacterium helveticum, Streptobacterium casei,* and *Streptococcus lactis.* This work was confirmed by Wood, Anderson, and Werkman (1937) in their study of the growth factors for the propionic as well as the lactic acid group. Snell and Strong (1939) found that *Lactobacillus casei,* strain No. 7469, requires riboflavin and that the amount of growth, as indicated by acid production, is proportional to the riboflavin present. Use was made of this fact in developing a method of assay for riboflavin in plant and animal tissue extracts.

EXPERIMENTAL PROCEDURE

The present work is a study of the riboflavin requirements of a large number of strains belonging to the genus *Lactobacillus* and a few strains belonging to the genera *Leuconostoc* and *Streptococcus.* For convenience and reference the original nomenclature, designating these cultures as received, was retained. The following cultures were studied: L-20, L-21, L-23, L-39, Scavano, and Wickerham labeled *Lactobacillus acidophilus;* L-4 and L-28 labeled *Bacterium brassicae fermentatae*; 8001, B-12, 7994, 7995, L-38, and L-32 labeled *Lactobacillus bulgaricus*; 7469, L-13, L-30, L-52, and L-37 labeled *L. casei*; L-3 and L-48 labeled *L. delbruckii*; 7999, L-32, L-43, L-80, and L-81 labeled *L. helveticus*; F-7-11, F-118-8, F-26 labeled *L. pentoaceticus,* L-53, F-36-6, F-102, L-14, L-29, labeled *L. plantarum;* and F-17-5 (*L. arabinosus*), L-46 (*L. beijerinckii*), L-35 (*Betabacterium breve*), L-25 (*Bacillus cucumeris fermentatae*), L-16 (*Lactobacillus fructovorans*), F-20 (*L. gayonii*), 7993 (*L. jugurt*), 8000 (*L. lactis*), L-40 (*Bacillus leichmanni*), L-36 (*Betabacterium longum*), L-15 (*Lactobacillus lycopersici*), L-17 (*Bacterium mannitopoeum*), F-19 (*Lactobacillus mannitopoeus*), L-54 (*Betabacterium rudensis*), F-11 (*Betabac-*

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terium vermiforme, Lactobacillus sp.), F-23 (*Lactobacillus* sp.), 688 (*Leuconostoc dextranicum*), 681 (*L. mesenterioides*), L-24 (*Bacterium gracile*), and one each of Group A (*Streptococcus pyogenes*), B (*S. mastitidis*), C ("Animal Pyogenes"), E and G streptococci; and two of Group D (*Streptococcus fecalis* and *S. zymogenes*) streptococci.

In general the assay method as described by Snell and Strong (1939) was used to study these organisms. All inoculations and incubations were carried out in a darkened room. Standard series of the riboflavin medium were set up and inoculated with washed cells grown originally in a broth medium containing 100 micrograms of riboflavin per liter. The amount of growth in response to the various levels of riboflavin was determined by titration using two drops of one per cent brom thymol blue as indicator.

Cultures were incubated at 37 or 30°C.(98.6 or 86°F.) dependent upon the optimum growth conditions of the various groups. Titrations of riboflavin series inoculated with strain No. 7469 (*Lactobacillus casei*) and No. 7993 were made after 72 hours of growth.

DATA AND DISCUSSION

Riboflavin in Metabolism of Certain Lactic Organisms: For convenience of discussion the strains of lactobacilli used may be roughly classed into several types, that is, the heat-tolerant milk types, the mouth and intestinal strains, the low-temperature milk types, the non-gas-producing plant type and the gas-producing plant type.

Six of the 13 strains of the first type including strains received as *Lactobacillus helveticus* and *L. bulgaricus* were identical in that no acid was produced (Fig. 1), even upon the addition of riboflavin to the base medium used. However, the remaining seven strains of this group responded to the presence of riboflavin. Cultures 7993 and L-38 (*Bacillus bulgaricus*) responded to riboflavin to a much greater degree than any of the other cultures, No. 7993 being especially outstanding in this respect.

The six strains from the mouth and intestine all required riboflavin for growth but each responded somewhat differently to its addition in increasing amounts (Fig. 2). The Scavano and Wickerham strains of *L. acidophilus* both produced less than three ml. of acid as a maximum while the other four cultures produced from seven to 10 ml. of acid in the riboflavin medium. The curves of the four higher acid producers ascend steeply at first and then level off abruptly, indicating that from 0.05 to 0.15 microgram of riboflavin per 10 ml. of medium was sufficient for maximum growth.

The titration curves for the five *Lactobacillus casei* cultures studied all show a fairly high maximum acid production (Fig. 3). One of the strains, L-13 (*Streptobacterium casei*) of Orla-Jensen, required no addition of riboflavin to the medium for maximum growth.

Of a group of non-gas-producing lactobacilli, including cultures received as *Lactobacillus leichmanni, L. beijerinckii, L. delbruckii, L. arabinosus,* and others, some failed to grow without the addition of riboflavin (Fig. 4) and responded moderately or very well to its addition,

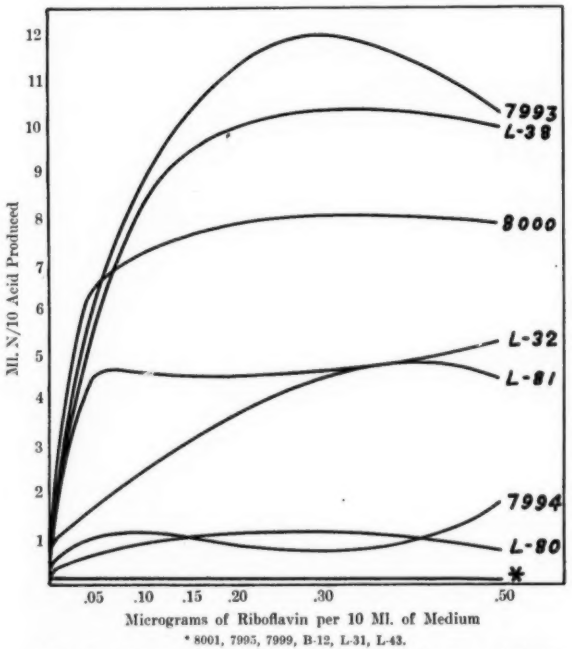

FIG. 1. Amounts of acid produced by strains originally designated as *Lactobacillus helveticus* 7999, L-81; *L. bulgaricus* 8001, B-12, 7994, 7995, L-38, L-32; *L. lactis* 8000; *L. delbruckii* L-31, L-43, L-80; and *L. jugurt* 7993, in response to various levels of riboflavin.

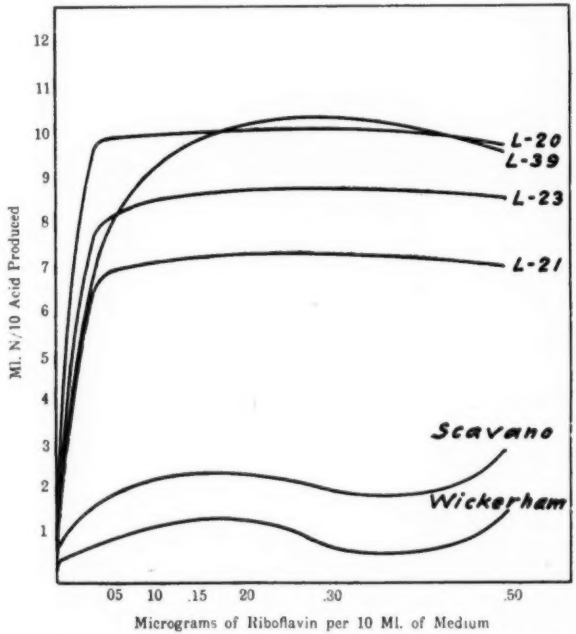

FIG. 2. Amounts of acid produced by strains originally designated as *Lactobacillus acidophilus* in response to various levels of riboflavin.

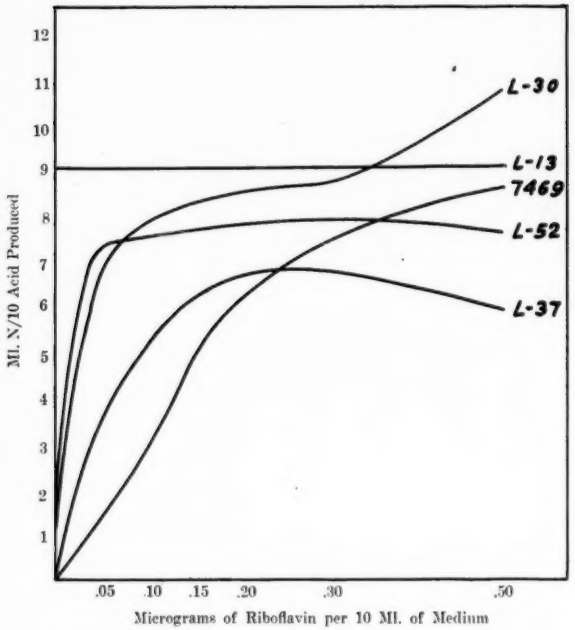

FIG. 3. Amounts of acid produced by strains originally designated as *Lactobacillus casei* in response to various levels of riboflavin.

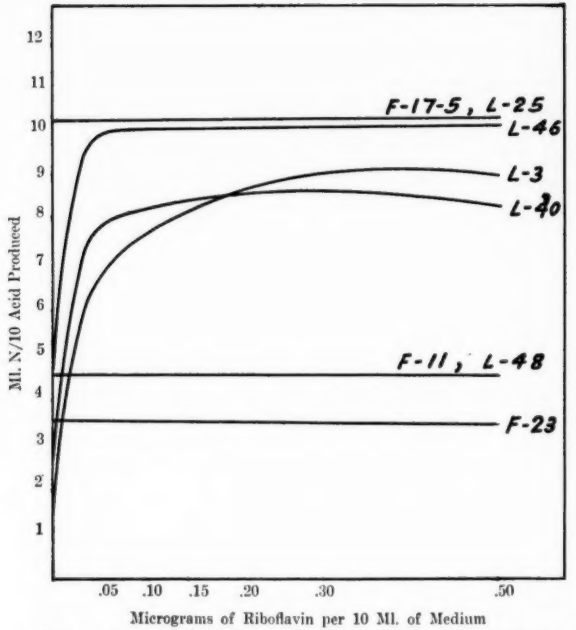

FIG. 4. Amounts of acid produced by strains originally designated as *Lactobacillus leichmanni* L-40, *L. delbruckii* L-3, L-48, *L. arabinosus* F-17-5, *Bacillus cucumeris fermentatae* L-25, *Lactobacillus sp.* F-11, F-23, and *L. beijerinckii* L-46 in response to various levels of riboflavin.

while other strains were not affected in their growth by the presence or absence of riboflavin.

Cultures of *Lactobacillus plantarum* (Fig. 5) tested either grew well in the absence of riboflavin or failed to grow regardless of the amount added to the medium with the exception of *Lactobacillus plantarum* var. *rudensis* which, although unable to grow in the riboflavin-free basal medium, produced increasing amounts of acid with its addition.

The titration curves of the gas-producing lactobacilli were generally high even in the absence of riboflavin or present only in concentrations less than 0.1 of a microgram per 10 c.c. Two strains, L-16 (*Lactobacillus fructovorans*) and L-24 (*Leuconostoc sp.*), however, did not grow at all in the medium even when increased amounts of riboflavin were added. The former is one of the most fastidious of the gas-producing types. Two other strains of *Leuconostoc* were stimulated by 0.05 to 0.10 microgram of riboflavin although both grew to some extent without riboflavin in the medium.

The effect of riboflavin upon the growth of seven streptococci was found to be strain variable (Fig. 6). The strain of *Streptococcus mastitidis* was the only one found to grow completely independently of added riboflavin, although strains of the Group D streptococci (*S. fecalis* and *S. zymogenes*) produced only small amounts of acid in the riboflavin-free basal medium. The strains of the two human and animal pathogens failed to grow appreciably in the medium with riboflavin added.

The *Lactobacillus* and *Leuconostoc* cultures could be separated as follows: (1) organisms failing to grow in the basal medium both with and without riboflavin; (2) organisms failing to grow in the basal medium alone and growing only very slightly when riboflavin is added; (3) organisms failing to grow in the basal medium but producing moderate to large amounts of acid with the addition of riboflavin; and (4) organisms growing in the basal medium and showing no increase in acid production with the addition of riboflavin. The streptococci studied could be divided in a similar manner. The more fastidious organisms of both the *Lactobacillus* and *Streptococcus* genera were found to be those which require factors for growth other than were present in the riboflavin medium used here, while the organisms cultivated readily on laboratory media were those which required little or no riboflavin in the medium for maximum acid production.

Six cultures considered to be strains of *Lactobacillus casei* and *L. helveticus* and to be very closely related, if not identical, were found to respond very differently to riboflavin. L-43 and L-31 (*L. helveticus*) were the only two which were alike in this respect, neither being able to grow in the medium even though riboflavin was added. The other four organisms at least must be considered as different strains, for their titration curves are very dissimilar. Thus it may be possible that the measure of the requirements of lactic acid bacteria, especially the lactobacilli, for riboflavin may subsequently serve as a character in the classification of these organisms. It would be indicated also that the conclusions of

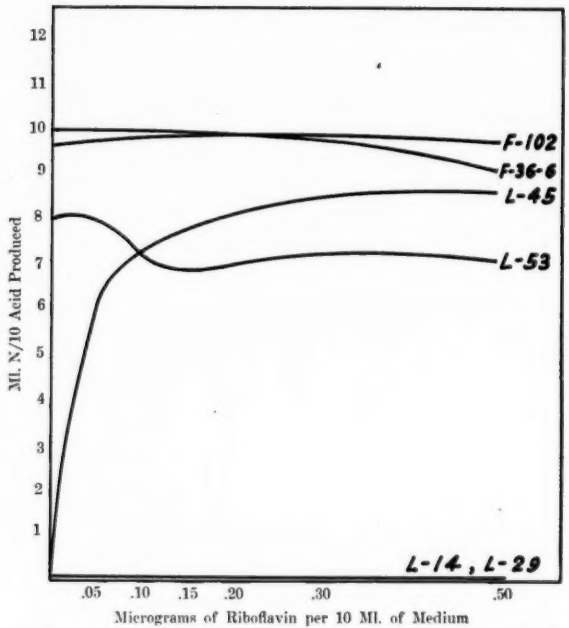

FIG. 5. Amounts of acid produced by strains originally designated as *Lactobacillus plantarum* in response to various levels of riboflavin.

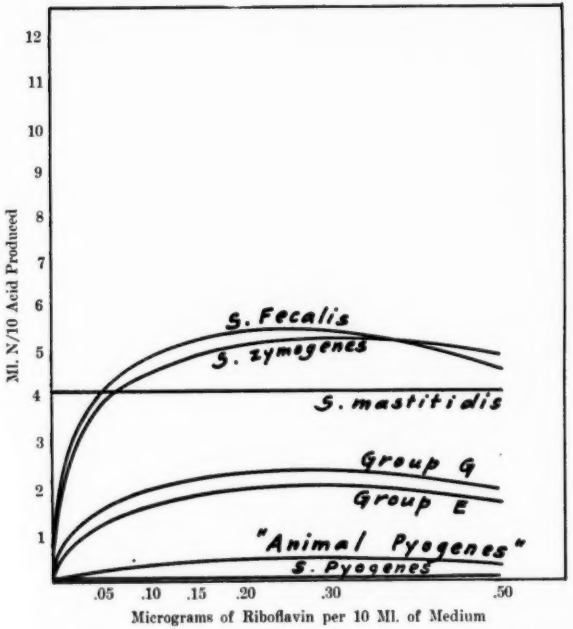

FIG. 6. Amounts of acid produced by certain strains of the genus *Streptococcus* in response to various levels of riboflavin.

Tittsler, Rocosa, and Whittier (1942) were substantiated in that Culture 7469 should be considered as closely related to the *L. casei* type.

New Organisms for Riboflavin Assay: Preliminary studies indicated that a satisfactory assay curve might be prepared with a *Lactobacillus* culture No. 7993 using a considerably lower range of riboflavin concentrations. This is more clearly indicated when the response of Culture 7993 (Fig. 7) is compared with the standard curve for growth response to riboflavin by Culture 7469 (*L. casei*) (Fig. 3).

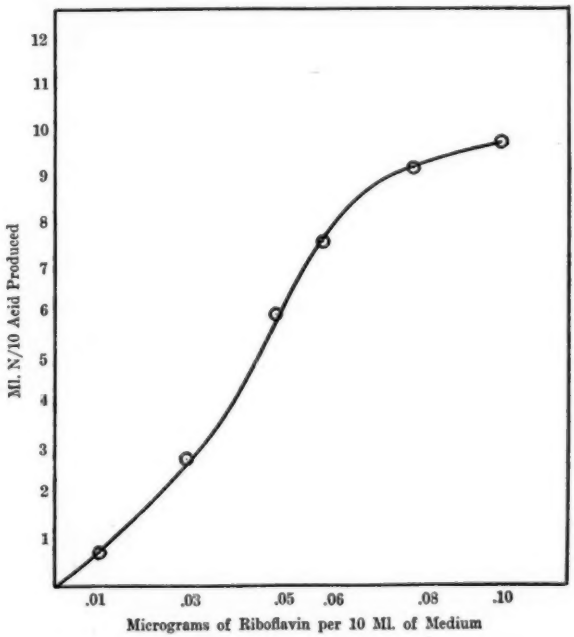

FIG. 7. Standard riboflavin titration curve for strain No. 7993 (*Lactobacillus*).

To determine the magnitude of variation among the titration values of Culture 7993 at each level of riboflavin, 10 identical series of tubes were set up to contain 0 and 0.01 to 0.10 microgram levels of the vitamin. The per cent of maximum variation from the average of 10 titration values obtained at each level (Table 1) is seen to be generally higher at the lower levels than at the top of the curve. Although the titration values do not check each other exactly at the different levels the variations are generally no greater than those which occur between duplicate assay tubes inoculated with Culture 7469 (*L. casei*).

Culture 7993 and also Culture 7469 (*L. casei*) were consistent upon repeated transfers through a stock culture medium prepared by adding 100 micrograms of riboflavin to 500 ml. of double-strength, riboflavin-free basal medium and diluting to one liter with distilled water.

TABLE 1

Variations in Amounts of Acid Produced by Strain 7993 in 10 Identical Tubes at Various Riboflavin Levels

Riboflavin per 10 ml. of medium	N/10 acid produced per 10 ml. of medium											Maximum variation from average	Maximum variation from average
	1	2	3	4	5	6	7	8	9	10	Av.		
μg.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*pct.*
0.00	0.45	0.55	0.60	0.50	0.75	0.55	0.60	0.50	0.45	0.65	0.56	0.19	33.9
0.01	0.70	1.00	0.80	0.80	0.90	1.30	0.80	0.70	1.15	1.05	0.92	0.38	41.3
0.03	3.35	3.50	3.25	3.20	3.55	3.55	3.15	3.50	3.20	2.80	3.30	0.50	20.0
0.05	5.60	5.55	5.40	5.15	6.00	5.40	5.65	6.10	5.30	5.75	5.60	0.50	8.9
0.06	7.10	6.15	6.70	7.55	7.50	5.80	6.85	7.30	6.75	8.10	6.98	1.12	16.9
0.10	9.05	10.85	10.00	9.70	9.70	9.90	9.95	9.80	9.90	.....	9.86	0.99	10.04

Comparative Studies When Culture 7469 (Lactobacillus casei) and Culture 7993 (Lactobacillus) Were Used as an Index in Riboflavin Assay: Riboflavin determinations were made on various fresh and dehydrated vegetable extracts to compare the accuracy of Culture 7993 (*L. jugurt*) with Culture 7469 (*L. casei*) in actual assay procedures. The vegetable extracts were prepared by autoclaving the material in distilled water for 15 minutes at 15 pounds pressure. It was found that an extract of proper concentration for assay with Culture 7469 (*L. casei*) had to be diluted five times to be of proper concentration for Culture 7993. For a limited series of tests it is indicated that Culture 7993 may show some promise as a test assay organism in those cases in which only minute amounts of riboflavin are present.

SUMMARY

A study of the riboflavin requirements of a large number of lactic acid bacteria indicates a wide but definite response to the presence of riboflavin in the medium. The lactic organisms could be grouped as follows: (1) organisms failing to grow in the basal medium both with and without riboflavin; (2) organisms failing to grow in the basal medium alone and growing only very slightly when riboflavin is added; (3) organisms failing to grow in the basal medium but producing moderate to large amounts of acid with the addition of riboflavin; and (4) organisms growing in the basal medium and showing no increase in acid production with the addition of riboflavin.

One culture, No. 7993 (*Lactobacillus*), was found which responded to smaller amounts of riboflavin than No. 7469 (*L. casei*), the present assay organism. Comparative studies of the two organisms indicate that Culture 7993 could be successfully used for assaying materials too low in riboflavin to be accurately assayed with Culture 7469 (*L. casei*), i.e., in the range of 0.01 to 0.10 microgram per five milliliters.

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VITAMIN A CONTENT OF SHEEP'S COLOSTRUM AND MILK[1]

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In Europe, especially in the mountain regions, sheep's milk is extensively used in the human dietary, and large quantities are manufactured into cheese. In this country very little sheep's milk is included in the human diet as such, but a significant amount of imported cheese made from sheep's milk is eaten. Zorn, Richter, and Wiener (1938) estimated the annual world production of sheep's milk for human consumption at 5,032 million liters. According to Morrison (1936) normal sheep's milk contains less water but more mineral matter and nearly twice as much protein and fat as cow's milk. Consequently its nutritive value should be significantly higher than that of cow's milk. However, relatively few studies have been made of the vitamin content of sheep's milk and a survey of the literature did not reveal any data concerning its vitamin A content. Accordingly, this study was inaugurated to determine the vitamin A content of sheep's colostrum and milk during early lactation.

EXPERIMENTAL PROCEDURE

Fifteen normal, well-developed animals were selected for this study from a well-bred flock of Hampshire ewes. During the summer and fall preceding the study they were maintained on an excellent pasture of lespedeza and native grasses. The ewes were housed about December 1, and for the next two months each received a daily ration of four pounds of soybean hay or soybean hay and whole soybeans. The day the lambs were born the ewes began to receive 1.5 pounds per head daily of a concentrate mixture of two parts of corn, two of oats, two of wheat bran, and one of cottonseed meal. All the ewes were born in January, February, and March and the lambs were born during February and early March. Thus the ewes were two or three years old when the samples of colostrum and milk were taken for study.

It was the first pregnancy for all the two-year-old ewes and for two of the three-year-old ewes, Nos. 46 and 982. Five of the three-year-old ewes, Nos. 8, 13, 28, 9, and 16, had produced one lamb at two years of age and No. 36 had two lambs when she was two years old. Detailed data concerning the ewes, their ages, number of lambs produced during the study, condition of the lambs at birth, and previous pregnancy, if any, are included (Table 1).

[1] Contribution No. 495 from the Massachusetts Agricultural Experiment Station.

The first sample of colostrum was collected in each case as soon as possible after the lamb was born. Thereafter samples of colostrum were taken in the morning. It was difficult to anticipate when the flow of colostrum would cease for the different ewes. Hence, whenever possible, samples were taken daily for a week or 10 days and afterwards at irregular intervals for varying periods after the lactation period was well established.

TABLE 1

Data Concerning Experimental Ewes

Ewe No.	Age in years	Number of lambs	Condition at birth	Previous pregnancy
2	2	1	Normal	None
15	2	1	Normal	None
29	2	2	Normal	None
3	2	2	One normal One dead	None
18	2	2	One normal One dead	None
37	2	2	Both dead	None
51	2	1	Dead	None
8	3	1	Normal	1 lamb at 2 yrs. old
13	3	2	Normal	1 lamb at 2 yrs. old
28	3	1	Normal	1 lamb at 2 yrs. old
9	3	2	One normal One dead	1 lamb at 2 yrs. old
16	3	2	Both dead	1 lamb at 2 yrs. old
36	3	2	Both dead	2 lambs at 2 yrs. old
46	3	1	Dead	None
982	3	1	Dead	None

The vitamin A content of the colostrum and milk was determined by a colorimetric method which included features described by Carr and Price (1926), Dyer and Wokes (1930), and Holmes, Tripp, and Satterfield (1936). The vitamin A was extracted from the milk by the method described by Dann (1933) in his investigation of vitamin A content of cow's milk and colostrum. The following procedure was used throughout. A 10-gm. sample was digested on a steam bath for one hour with 10 c.c. of 66-per cent alcoholic KOH. The flask was cooled and its contents poured into a separatory funnel together with 10 c.c. of alcohol and extracted with 30 c.c. of ethyl ether. The ether was washed three times with 50-c.c. portions of distilled water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in chloroform which was adjusted to a volume whereby 0.1 to 0.5 c.c. would give a reading within the limits of the tintometer. The chloroform solution was pipetted into a tintometer cell and a saturated solution of antimony trichloride in chloroform was added until the total volume was 2.5 c.c. The depth of the blue color produced was compared in a Lovibond tintometer with that of standard color glasses. The results of the individual assays of colostrum and milk are reported (Table 2).

DISCUSSION

The first series of samples of colostrum obtained from the two-year-old ewes varied in vitamin A content from 2.87 to 21.90 and averaged 9.65 Lovibond units per gm. On the second day the average vitamin A content of the colostrum was essentially the same for the two-year-old ewes, being 9.18 Lovibond units per gm. The vitamin A values obtained for the colostrum from the three-year-old ewes on the first day of lactation varied from 3.08 to 16.73 gm., with an average of 10.01 units per gm., but on the second day the average had dropped to 5.92 Lovibond units per gm.

Since their lambs were born dead, samples were collected regularly from the two-year-old ewes, Nos. 37 and 51, and the three-year-old ewe, No. 36, for only two days following parturition. The average values on the third day of lactation were 4.88 for the other four two-year-old ewes and 4.39 for the seven three-year-old ewes. The values for the fourth day were 4.95 for the two-year-old ewes, and 4.01 for the three-year-old ewes.

By the end of the first week the vitamin A content of the milk had dropped to 0.81 Lovibond units per gm. for the two-year-old ewes and 5.64 Lovibond units per gm. for the three-year-old ewes. The high average values obtained for the three-year-old ewes were due to the unusually high values obtained for ewe No. 46. The first sample of colostrum obtained from this ewe showed 29.1 Lovibond units per gm. immediately after the lamb was born. Several hours later on the same day the value had dropped to 16.73, but high values were obtained for more than a week. This abnormal situation may have been due to the presence of colostrum which was not removed from the udder as would have been the case if her lamb had been born alive and suckled normally.

In addition to the results obtained for the vitamin A content of samples from the individual ewes, Table 2 contains average values for the vitamin A content of the milk collected only from those ewes whose lambs grew to maturity. In all probability these latter values are more nearly typical of the vitamin A content of ewe's colostrum and milk than those which include data from ewes which produced dead lambs. Judged by these data, the lamb removes the colostrum from the ewe's udder quite rapidly, for by the third day of lactation the vitamin A content had dropped from an initial 10.11 to 3.98 Lovibond units per gm., and by the sixth day of lactation the vitamin A content was 1.03 units per gm. or about one-tenth that of the initial colostrum. This rate of decrease is practically identical with that reported by Barron (1942) from a study with 13 Dorset Horn cross Border-Leicester ewes. Barron obtained average vitamin A values of 56.1 units one hour after paturition, 21.7 units at 48 hours, 10 units after four days of lactation, 5.8 units after six days, 4.8 units after eight days, and 5.6 Lovibond units per gm. after 10 days of lactation. The decrease of vitamin A content of ewe's colostrum and milk during early lactation is quite similar to that of cows, for Dann (1933) reports that, independent of the season of the year, the vitamin A concentration of colostrum may be from 10 to 100 times larger than in subsequent milk from the same cow. Dann (1933) also found that the vitamin content of colostrum and early milk of heifers was higher than that of

TABLE 2

Vitamin A Content[1] of Sheep's Colostrum and Milk

Ewe No. and age	Lactation period (days)															
	1	2	3	4	5	6	7	8	9	10	14	15	17	20	21	22
2 yrs. old																
2	9.66	9.94	3.15	1.47	0.92	0.26	.....	.....	0.28	0.39	.....	.....	.....	.....	.....	.....
15	2.87	.....	.....	.....	0.85	.....	.....	0.93	.....	.....	.....	0.33	.....	.....	.....	.....
29	7.25	6.31	4.97	0.93	3.09	.....	0.66	.....	.....	.....	.....	.....	0.30	.....	.....	.....
3	21.90	8.44	7.25	13.21	5.51	2.01	0.96	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	.....	7.27	4.15	4.20	3.62	0.29	.....	.....	.....	.....	0.44	.....	.....	.....	.....	.....
37	4.64	7.92	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.47	.....	.....
51	11.55	15.20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.52	.....	.....	.....
Average for 2 yrs. old	9.65	9.18	4.88	4.95	2.80	0.85	0.81	0.93	0.28	0.39	0.44	0.33	1.41	0.47	.....	.....
3 yrs. old																
8	13.11	5.06	1.91	0.86	.....	0.29	.....	.....	.....	.....	.....	0.24	.....	.....	.....	0.25
13	10.05	.....	3.15	1.28	1.94	1.27	.....	.....	.....	.....	0.64	.....	.....	0.29	.....	.....
28	8.92	6.35	2.46	1.45	3.44	0.66	0.78	.....	.....	0.55	.....	.....	.....	0.25	.....	.....
9	7.08	4.78	4.77	3.54	2.24	2.42	2.12	0.59	.....	.....	.....	.....	1.19	.....	.....	.....
16	3.08	2.02	1.08	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
36	11.11	2.19	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.45	.....
46	16.73	13.48	13.08	12.82	22.77	17.03	15.32	14.41	8.14	.....	.....	.....	.....	.....	.....	.....
982	.....	7.55	4.29	4.11	2.09	.....	.....	.....	1.57	.....	.....	.....	.....	.....	.....	.....
Average for 3 yrs. old	10.01	5.92	4.39	4.01	6.50	4.33	6.07	7.50	4.85	0.55	0.64	0.24	1.19	0.27	1.45	0.25
Average for all	9.84	7.42	4.57	4.39	4.65	3.03	3.97	5.31	3.33	0.47	0.54	0.29	1.34	0.34	1.45	0.25
Average for 2 yrs. old[2]	10.42	7.99	4.88	4.95	2.80	0.85	0.81	0.93	0.28	0.39	0.44	0.33	0.30	.....	.....	.....
Average for 3 yrs. old[2]	9.79	5.40	3.07	1.78	2.54	1.16	1.45	0.59	.....	0.55	0.64	0.24	1.19	0.27	.....	0.25
Average for all[2]	10.11	6.88	3.98	3.37	2.70	1.03	1.13	0.76	0.28	0.47	0.54	0.29	0.75	0.27	.....	0.25

[1] Lovibond Blue units per gram. [2] Data for ewes that produced lambs which grew to maturity.

cows. In this study the vitamin A content of the colostrum and early milk of the two-year-old ewes was some higher than that of the three-year-old ewes.

One may question why 12 of the 22 lambs from supposedly normal ewes were born dead. It is possible that the soybeans were a prominent factor, if not the sole cause, of this high mortality. In 1931 Hostetler and Foster (1943) observed that nursing ewes fed mature soybean hay developed abnormal symptoms. The majority of their lambs died and many of the ewes developed inflamed and congested udders. They also stated that farmers in their locality experienced similar difficulties with ewes, swine, and cows. Wilbur, Hilton, and Hauge (1935); Hauge, Wilbur, and Hilton (1937); and Hauge (1937) reported that soybeans contain a factor which interferes with the transference of vitamin A (carotene) potency of the ration to the milk fat secreted by cows. Sharpless (1938) found that rats readily developed goiter when fed a ration containing a large proportion of unprocessed soybean flour and that the enlarged thyroid glands were hyperplastic with little or no colloid. Wilgus (1940) stated that soybean oil meal contains a goitrogenic factor which is insoluble in chloroform, hexane, ether, acetone, or alcohol. Sharpless, Pearsons, and Prato (1939) observed that rats fed a ration of unprocessed soybean flour (75 per cent), yeast, sugar, butterfat, sodium chloride, and calcium carbonate for a seven-weeks experimental period developed enlarged thyroid glands which were four times the normal size. Wilgus, Gassner, Patton, and Gustavson (1941) stated that the intense goitrogenicity of soybeans is evident by the enlargement of chicks' thyroid glands to eight or 10 times the normal size.

SUMMARY

Normal, well-developed two-year and three-year-old Hampshire ewes were selected for this study. During the summer and fall preceding the study they were maintained on an excellent pasture of lespedeza and native grasses. In December and January, they received four pounds of soybean hay or soybean hay and soybeans. When their lambs were born in February and early March, each received in addition 1.5 pounds daily of a mixture of corn, oats, wheat bran, and cottonseed meal.

The first sample of colostrum was collected as soon as possible after parturition. Thereafter, samples were collected each morning for a week or more and subsequently at less frequent intervals. The samples were assayed by the colorimetric method for their vitamin A content.

The average vitamin A content of the colostrum and milk obtained from ewes that produced lambs which grew to maturity was 10.11, 6.88, 3.98, 3.37, 2.70, 1.03, and 1.10 Lovibond units per gm., respectively, for the first seven days of lactation. During the second and third weeks of lactation, the vitamin A content of the milk was lower. The vitamin A content of the colostrum and early milk of the two-year-old ewes was slightly higher than that of the older animals.

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IRON CONTENT OF MARKET MILK

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According to current tables of food composition, the iron content of milk is two milligrams per kilogram.[1] That this value might be too high first occurred to the writer after finding that a sample of milk which was analyzed as part of a dietary study contained only a fraction of that amount. This led to a survey of the literature to discover the values found by recent analysts and eventually to the analyses reported here.

The most extensive study of the iron content of milk reported in the last 10 years is the one made by the Rowett Research Institute of England (1934); the average for 220 samples was 0.89 mg. per liter, with a range from 0.27 to 1.4 mg. per liter. This value, however, may not be representative of the milk on the market in all other countries. Even if milk as it is secreted from the cow were the same throughout the world, it is quite possible that it would pick up more iron during handling in some places than in others.

In this country during the last 10 years, results of analyses of iron content of milk have been reported by at least eight workers (Table 1). In most cases these analyses were incidental to other studies. In only one study, that by Toscani and Reznikoff (1934), is the value reported as high as the one in the current tables of food composition. In the other seven studies the values range from 0.34 to 0.96 mg. per kg. or only about one-third of that value. Since most of these studies were made on only one to three samples, and since the only extensive one [Krauss and Washburn (1936)] was not made on regular market milk but on milk drawn into enamel containers, they do not yield enough information to lead to the establishment of a new value. They do, however, indicate the need for one. It was to fulfill this need that the present study was undertaken.

EXPERIMENTAL PROCEDURE

Sixty-one bottles of market milk distributed by 25 companies were used. These samples were chosen so as to include milk of various bacterial counts within the Grade-A classification and of various butterfat contents. They were from large and small companies in various parts of the United States, and they were taken at different seasons. All were pasteurized and a few were homogenized and had vitamin D added. Some were packaged in glass bottles and some in paper cartons.

For each analysis, triplicate samples of whole milk were pipetted from the original market container into platinum dishes, dry-ashed, and iron was determined by Stugart's thiocyanate method (1931). The depth of

[1] Sherman, H. C., 1941. Chemistry of Food and Nutrition. The MacMillan Company, New York, N. Y.

color of the iron salt was determined in a Coleman Universal Spectrophotometer. Great care was taken to prevent contamination throughout the entire process.

The results of the analyses on samples collected in Chicago, in other cities throughout the United States, and in a rural area are given (Table 2). The milk on the Chicago market will be considered first. The milk of ten distributors supplying 11 products, ranged in value from 0.248 to 0.376 mg. per kg. with an even distribution along the range. The mean was 0.304 mg. per kg. This, however, does not take into account the fact that some distributors handle much larger volumes of milk than others. The products of the two largest distributors, A and B, who supply approximately half of the milk in the Chicago marketing area were the two containing the least iron.

TABLE 1

Iron Content of Milk According to Analyses Made in the United States Since 1933

Author	Number of samples	Analytical method	Iron content
Toscani and Rezinkoff (1934)	3	Thiocyanate (Elvehjem)	1.3-2.6 mg. per kg.
Leavell and Ellis (1934)	3	Mercapto—acetic acid	0.6-0.75 mg. per liter
Farrar (1935)	1	Thiocyanate (Farrar)	0.5 mg. per kg.
Ascham (1935)	1	Thiocyanate (Stugart)	0.96 mg. per kg.
Krauss and Washburn (1936)	9	Thiocyanate (Stugart)	0.34-0.43[1] mg. per liter
Stearns and Stinger (1937)	3	Thiocyanate (Stugart)	0.38-0.72 mg. per liter
Elvehjem *et al.* (1937)	1	Thiocyanate (Elvehjem)	0.50[2] mg. per liter
Roberts *et al.* (1940)	1	Thiocyanate (Stugart)	0.42 mg. per kg.

[1] Drawn into enamel vessels. [2] Certified milk.

The question then arose as to whether or not the low iron content of the milk on the Chicago market is typical of the rest of the country. Samples of 13 milks were brought in by airplane [2] from 10 cities widely scattered throughout the United States. The range of 0.114 to 0.650 mg. per kg. (Table 2) was wider than for the Chicago milks but the distribution within the range was similar. The mean of 0.330 mg. per kg. was close to that of the Chicago milks.

Since all the communities from which milk had been obtained were large ones, it seemed advisable to secure some from a rural area. Samples were procured, therefore, from three distributors in Miami County, Ohio. These samples were chosen so as to be representative of the nine distributors in the county and, also, of the six distributors who supplied milk for Troy, Ohio, a town of 9,000 population, located within the county. The plants of these distributors were in Troy and supplied about half of the milk of that community as well as much of the milk in the small towns nearby. The regular milk of these companies contained 0.257, 0.310, and 0.446 mg. per kg. (Table 2). The one containing the most iron was

[2] Most of these samples were supplied by the courtesy of the United Air Lines. This made it possible to obtain the milk samples before souring made accurate sampling difficult.

from the company distributing the smallest volume of milk. These values are similar to those in the large cities.

DISCUSSION

These analyses indicate that the iron content of the milk in Chicago, in several of the large cities in the United States, and in one rural area is similar. In fact, the iron content seems to vary, not with location, but with the individual distributors. It would be interesting to know why the milk marketed by some distributors has twice as much iron in it as that of others. This variation may be due to an actual difference in the iron content of the milk as it is secreted by the cow or to contamination in handling or to both. The possibility of variation in the milk as secreted will be considered first.

That the iron content of milk is altered by variations in the iron content of the ration fed to the cows has been disproved. After trebling the iron content of the ration of 10 cows, Krauss and Washburn (1936) found no increase in the iron content of the milk. Elvehjem, Herrin, and Hart (1926) also found no increase in the iron content of milk following an increase in intake.

Since the protein content of Guernsey milk is 17 per cent greater than Holstein, it seemed possible that differences in breed might account for some variation in iron content. Samples of pure Holstein and pure Guernsey milk were obtained before pasteurization.[3] Two bottles of Guernsey milk contained an average of 0.380 mg. per kg., and two bottles of Holstein contained an average of 0.337 mg. per kg. The Guernsey was 13 per cent higher in iron content. Since this is only one case and since the error of the method may be as high as 10 per cent when such minute amounts of iron are concerned, about all that can be concluded is that Guernsey milk contains a little more iron than Holstein but so little that it makes no important difference in dietary calculations. That this conclusion is correct is corroborated by the analyses of the milk of three distributors who were marketing both a mixed-breed milk and a pure Guernsey milk. In the cases of Distributors A and W the mixed-breed milk contained less iron than the Guernsey, but very little less (Table 2); from the case of Company Q it is impossible to draw any evidence because the iron content of the two bottles of mixed-breed milk was so different.

If there were a seasonal variation in the iron content of milk, this might result from an actual change in the milk produced by the cow. In this study the milk samples from Chicago were taken at different seasons but no evidence of any relationship to season appeared.

There is also the possibility that the variations in the iron content of milk may be due to iron dissolved from the equipment with which milk comes in contact in the process of handling it for the market. Krauss and Washburn (1936) drew milk into an enamel container and then analyzed it. Using the same method as the one employed in this study, they found 0.34 to 0.43 mg. per liter. This is close to the amount of iron found

[3] This milk was supplied by the courtesy of the Farmer's Cooperative Dairy, Troy, Ohio.

TABLE 2

Iron Content of Market Milk

Location	Distributor	Bottle	Date	Iron content	Av. iron content
				mg./kg.	*mg./kg.*
Chicago, Ill.	A	1	Dec. '42	.241	.....
	A	2	Feb. '42	.255	.248
	A (Guernsey)	1	July '43	.256	.....
	A (Guernsey)	2	July '43	.261	.258
	B	1	July 14, '43	.244	.....
	B	2	Feb. '42	.265	.....
	B	3	July 17, '43	.294	.....
	B	4	Jan. '42	.301	.276
	C	1	July '43	.278	.....
	C	2	July '43	.284	.281
	D	1	July 8, '43	.287	.....
	D	2	Jan. '42	.296	.....
	D	3	July 2, '43	.363	.291
	E	1	Dec. '42	.283	.....
	E	2	July '43	.303	.....
	E	3	Feb. '42	.324	.303
	F	1	Jan. '42	.295	.....
	F	2	Sept. '43	.331	.313
	G	1	Sept. '43	.304	.....
	G	2	July '43	.315	.....
	G	3	July '43	.327	.315
	H	1	Dec. '42	.325	.....
	H	2	Jan. '42	.336	.330
	I	1	Feb. '42	.347	.....
	I	2	Feb. '42	.356	.351
	J	1	July '43	.366	.....
	J	2	Feb. '42	.387	.376
Average	....	....	.....	.....	.304
Urban (outside Chicago)	....	....	.....	.....[1]	.....
Lincoln, Neb.	K	1	Apr. 7, '42	.075	.....
	K	2	Apr. 23, '42	.151[1]	.....
	K	3	Apr. 30, '42	.117[1]	.114
	L	1	May '42	.235[1]	.235
New York, N. Y.	M	1	Mar. '42	.248	.....
	M	2	Mar. '42	.254	.251
St. Louis, Mo.	N	1	Feb. '42	.286	.....
	N	2	Feb. '42	.299	.292
Salt Lake City, Utah	O	1	Mar. '42	.290	.....
	O	2	Mar. '42	.305	.297
Boston, Mass.	P	1	Mar. '42	.315	.....
	P	2	Mar. '42	.316	.315
Jacksonville, Fla.	Q	1	Apr. '42	.316	.316
	Q	2	July '43	.545[2]	.....
	Q (Guernsey)	1	July '43	.418	.....
	Q (Guernsey)	2	Apr. '42	.501	.459
Los Angeles, Calif.	R	1	Apr. '42	.318	.....
	R	2	Apr. '42	.318	.318
San Francisco, Calif.	S	1	Apr. '42	.334	.....
	S	2	Apr. '42	.337	.335
New York, N. Y.	T	1	Feb. '42	.340	.....
	T	2	Feb. '42	.356	.348
Portland, Ore.	U	1	Apr. '42	.350	.....
	U	2	Apr. '42	.368	.359
Denver, Colo.	V	1	Mar. '42	.620	.....
	V	2	Mar. '42	.681	.650
Average	....	....	.....	.....	.330
Rural					
Miami County, Ohio	W	1	Jan. '43	.251	.....
	W	2	Jan. '43	.264	.257
	W (Guernsey)	1	Jan. '43	.273	.....
	W (Guernsey)	2	Jan. '43	.293	.283
	X	1	Sept. '43	.292	.....
	X	2	Sept. '43	.329	.310
	Y	1	Sept. '43	.401	.....
	Y	2	Sept. '43	.492	.446
Average	....	....	.....	.....	.324
Average for all distributors	....	....	.....	.....	.319

[1] These analyses were made by Dr. Ruth Leverton at the University of Nebraska. [2] It is likely that this bottle became contaminated.

in the present study with one exception. Therefore, most of the milks analyzed in this study apparently had not picked up much, if any, iron. The reason for this is that the linings of the equipment used in dairies and milk plants are made of very hard and resistant metals or of glass.

It is possible that some market milk contains less iron than when drawn from the cows because iron has been removed in the pasteurization process. This possibility was suggested by the differences in iron content of the samples of unpasteurized Guernsey and Holstein milk supplied by Distributor X for the study of breed differences and the samples of regular pasteurized milk supplied by the same distributor. Since the regular, mixed-breed milk was about half Guernsey and half Holstein, values for them may be averaged to give 0.358 mg. per kg. before pasteurization while the pasteurized milk contained 0.310 mg. per kg.

The purpose of this study was to decide what value should be selected as representative of the average iron content of the milk on the markets in this country. The combined results of the milks from Chicago, the other cities, and the rural area should furnish a basis for such a decision. The range was from 0.114 to 0.650 mg. per kg. with a mean of 0.319 mg. per kg. The median fell between 0.310 and 0.313 mg. per kg. and the modal group was 0.310 to 0.320 mg. per kg. The volume of milk distributed by each company is not taken into consideration on the above values. Since the milk of the largest companies tends to be low in iron, it is probably justifiable to select 0.30 mg. per kg. as a good average for all market milk.

Why is this value so much lower than the one found in the tables of food composition? There are two possibilities. One is that there has been an improvement in the method of analysis. The method employed in the most recent work, and in this study, was the thiocyanate method as modified by Stugart and published in 1931. For a detailed discussion of the advantages of this method the reader is referred to the article by Stugart. The other possibility is that some market milks actually contained more iron a decade or two ago, when the value of 2.0 mg. per kg. was adopted, than they do today because the metals used for vessels in all dairies and milk plants at that time may not have been of a highly resistant type.

The question might also be raised as to why the value found in the present study is somewhat lower than most of the values reported in the recent literature. Krauss and Washburn (1936), the only authors reporting values as low as those in the present study and the only ones reporting more than three analyses, state in a footnote that the first two values of approximately 0.5 mg. per liter which they reported are probably too high, since similar samples analyzed after "further refinements in technique"[4] contained only 0.36 mg. per liter. The same thing happened in the present study. The first three analyses yielded values in the neighborhood of 0.5 mg. per kg. These values were discarded because later, after further precautions against contamination were taken, these same milks under similar conditions of sampling yielded lower values.

[4] Krauss and Washburn (1936) p. 249.

SUMMARY

Sixty-one bottles of market milk from 25 companies in 12 communities were analyzed for iron by Stugart's thiocyanate method. The iron content ranged from 0.114 to 0.650 mg. per kg. The mean was 0.319 mg. per kg. Probably the best value to adopt for average market milk is 0.3 mg. per kg. This is only one-seventh of the value given in current tables of food composition.

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INFLUENCE OF TEMPERATURE ON CHANGES IN STORAGE EGGS AS MEASURED BY RADIO-FREQUENCY CONDUCTIVITY

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It has been shown that yolk and albumen of chicken eggs undergo with age rapid changes in their physical and chemical properties. There is a transference of water from the albumen to the yolk and back; loss in water first from the albumen and then also from the yolk; equilibration in osmotic pressure between the yolk and albumen — Smith and Shepherd (1931); increase in hydrogen-ion concentration; and formation of ammonia—Romanoff (1940). The rate of physical and chemical breakdown has been considered to be influenced greatly by temperature, somewhat by humidity, and possibly by several other environmental factors.

Since all these changes, which are indicative both of age and deterioration of eggs, so far have been recorded on dissected eggs, there is a distinct need for a method which would permit successive observations of physicochemical activity of the same egg without its dissection. The radio-frequency field is one among the methods that may offer such possibilities. This has already been demonstrated in differentiation of fertility in fresh intact eggs by high-frequency conductivity and dielectric effect—Romanoff and Cottrell (1939) and Romanoff and Frank (1941).

EXPERIMENTAL PROCEDURE

White Leghorn (*Gallus domesticus*) eggs of uniform weight, shape, and shell texture were used. They were divided in equal lots and kept at 5, 13, and 21°C. (41, 55.4, and 69.8°F.) with relative humidity of about 55 per cent. Six eggs from each group were measured individually at weekly intervals, during a 10-week period, for relative conductivity at a frequency of about 12 megacycles, by the method previously described—Romanoff and Cottrell (1939) and Romanoff and Frank (1941). At the end of the experimental period all eggs were examined to determine their relative marketable value for human consumption.

RESULTS AND DISCUSSION

It was observed (Fig. 1) that radio-frequency conductivity increases with the age of the egg. This increase was very rapid in eggs held at room temperature of 21°C. and relatively slow in those held in a refrigerator at 5°C. At 13°C. the increase was intermediate between the two extreme groups.

At the end of the 10 weeks of storage all eggs were graded on the basis of their appearance (determined by candling) and on internal examination. This observation showed that radio-frequency conductivity follows the deterioration rate of eggs with aging, and the rate was increased with

an increase in temperature. Eggs held at a temperature of 21°C. passed rapidly from the fresh state to a low market grade and then to an inedible condition, while at the lower temperature of 5°C. the change in quality of eggs was slow, particularly during the first month of storage.

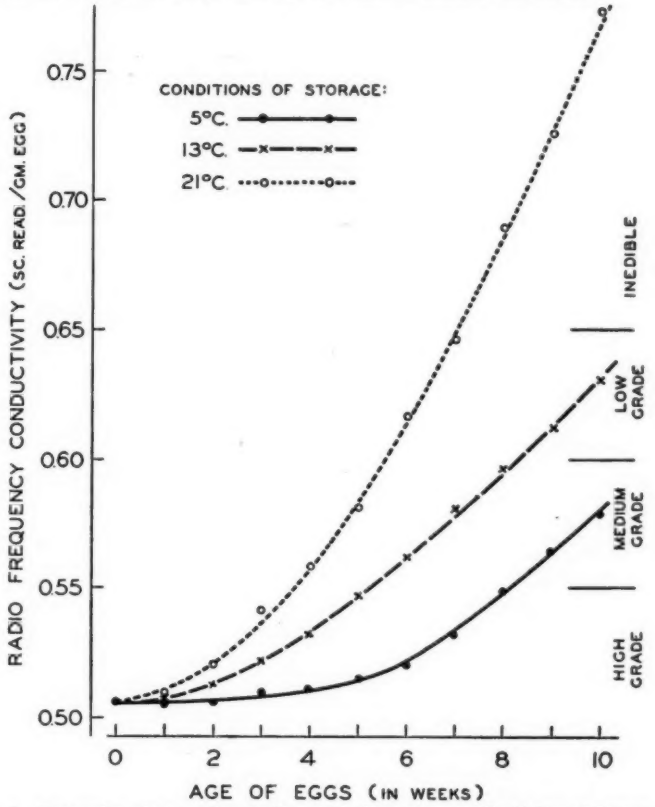

FIG. 1. Successive changes in relative radio-frequency conductivity of intact chicken eggs kept under various storage conditions. (The approximate market grades of these eggs at the end of the 10-week period are indicated.)

The results of these observations have two important aspects: theoretical and economic. From the theoretical point of view it is of interest to explain the underlying cause of changes in radio-frequency conductivity, while from the economic point of view the possibility may be seen of the use of this new technic in studies of successive chemical deterioration of intact eggs.

The increase in radio-frequency conductivity of eggs with age could not be attributed entirely to the loss in water through evaporation, because the loss in weight of these eggs was found to be linear—Hall and Romanoff (1943)—while the changes in conductivity follow a more or less general exponential curve of many physicochemical changes within the egg.

After laying, the changes in radio-frequency conductivity of egg yolk and albumen still remain to be determined. It has been shown that in fresh eggs under the influence of fertilization—Romanoff and Frank (1941)—albumen undergoes marked change, at least during the uterine period. A slight increase in water content of the yolk during its passage through oviduct—Romanoff (1931)—presumably has no influence on conductivity.

The observations, showing that the rate of increase in radio-frequency conductivity is closely related to deterioration in eggs, are of particular interest to the food industry. Considering the relative inefficiency of the present primitive method of grading of market eggs by candling, further studies of the use of high-frequency field may offer a better method and add to the accuracy in evaluation of quality of table eggs irrespective of their age or condition of storage.

CONCLUSIONS

Radio-frequency conductivity of intact hen's eggs increases with their age.

The rate of increase in conductivity is greatly influenced by holding at higher temperatures.

The conductivity of eggs at all ages is directly related to the degree of their deterioration.

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CHANGES IN HISTOLOGICAL STRUCTURE AND PALATABILITY OF BEEF DURING STORAGE [1]

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The effects of storage on the palatability of beef have been investigated by many workers, but very few studies have been made in which the histological changes during the onset and dissolution of rigor and subsequent ripening were investigated. A study of such changes may lead to a better understanding of the factors causing the increased palatability claimed for beef which has been ripened. The work reported herein was done on the voluntary, cross-striated muscles of beef, since these are the ones chiefly used for food.

Mangold (1921, 1922a,b) studied the onset of rigor in mice, rats, and rabbits. He found that, in general, an isolated muscle went into rigor faster, took longer to attain maximum stiffness, but began to lose rigor more quickly than did the entire body of the animal. The muscles in rigor were approximately 67 per cent stiffer (more resistant to pressure) than they were immediately after death.

Smith (1939) found that the onset of rigor in rabbit muscle increased the modulus of elasticity from 700-3,000 to about 10,000. The time of onset of rigor in normal muscle varied from zero to seven hours or more, with violent activity immediately before killing hastening its appearance.

One of the common observations in microscopic studies of rigor is the variable response of the fibers, Carey (1940). Some fibers develop the characteristic zones of contraction and rarefaction, others exhibit a wavy or kinked appearance which is interpreted by Carey to indicate that these fibers are not under tension but are passively retracted by the contraction of the noded fibers. The appearance of the contraction nodes also varies with the means employed in inducing rigor. In the stretched regions between the nodes in any given fiber, rigor increases the prominence of the longitudinal striations. There is also some slipping of the fibrils, so that the bands which give the cross-striated appearance are no longer perfectly aligned. This gives rise to irregularities in these striations, such as Vernier effects, zigzags, and diagonal stripes.

Hanson, Stewart, and Lowe (1942) found that with dissolution of rigor thinning of the cell contents occurred at random points in scattered broiler muscle fibers. Next sharp breaks or ruptures appeared. These were followed by the development of long areas in which the striated structure of the fiber had disintegrated. The disintegrated areas became larger and more numerous as the storage period was increased. Dissolution of rigor

[1] Part of a dissertation submitted by Pauline Paul to the faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Miss Paul is now with Swift and Company, Research Laboratory, Chicago 9, Illinois.

occurred earlier in breast than in thigh muscles and earlier in some birds than in others. These changes in structure of the fiber were correlated with the changes in tenderness of muscle with dissolution of rigor.

The studies on storage of meat prior to 1909 have been summarized by Emmett and Grindley (1909). Since that time several studies have been made, centering chiefly on chemical and palatability changes.

Hoagland, McBride, and Powick (1917), Lehmann (1907), Steiner (1938), Griswold and Wharton (1941), Beard (1942), and Moran and Smith (1929) agreed that one of the most important palatability changes during ripening of beef was increased tenderness. However, there was some disagreement concerning the magnitude of the change in any given time, probably arising from differences in the experimental materials and conditions. The consensus seems to be that beef should be held in storage from 10 days to two weeks to allow for an increase in tenderness. Beyond this point, flavor changes occur which may be desirable or undesirable.

Moran and Smith (1929) attributed the increase in tenderness which occurred when beef was hung to chemical change in the proteins of the muscle substance and the protein, collagen, of the connective tissues. They thought the alteration in the proteins of the muscle fiber was the most important change in the first few days after death, for this caused the disappearance of rigor. Several days after death, at a time depending mainly upon temperature, rigor was completely resolved, and further increase in tenderness was due mainly to the softening and swelling of the collagen.

Steiner (1938, 1939) stated that the decrease in toughness of beef during the first four or five days of storage was caused chiefly by changes in the muscle fibers. Changes in the connective tissue, by autolytic or other means, affect the tenderness of beef only after longer storage, 20 to 30 days. Since the increase in tenderness of beef was not great after 20 to 30 days of storage, Steiner thought the role of the connective tissue was a minor one in tenderizing beef by storage.

Lehmann (1907), Mitchell, Hamilton, and Haines (1928), and Mackintosh, Hall, and Vail (1936) found a high correlation between connective tissue content and tenderness of beef.

In the cooking process there are two opposing factors affecting tenderness—one is the change in connective tissue, the other the change in the muscle-fiber proteins. The elastic tissue is not changed by the normal cooking processes, but part of the collagen is hydrolyzed, the extent of hydrolysis increasing with longer cooking time. This hydrolysis increases the tenderness of the meat. Satorius and Child (1938) found, however, that the tenderness decreased if beef was cooked to internal temperatures above 67°C.(152.6°F.). This they attributed to the toughening effect of heat in coagulating and hardening the muscle-fiber proteins.

EXPERIMENTAL PROCEDURE

The beef used in this study consisted of a pair of rounds and a pair of psoas major muscles from a "good" grade, yearling steer. The muscles utilized were the semitendinosus, semimembranosus, biceps femoris, the vastus group (lateralis, intermedius, and medius), gastrocnemius, adductor,

and psoas major. Each of the first four muscles was divided into six roasts, three from the right and three from the left side of the animal. The last three muscles were combined into one group. It had been originally planned to test the same muscles from several animals, but the rationing program made it impractical to use meat from more than one animal.

The storage times employed were zero, one, two, four, nine, and 18 days. The roasts with no storage were cooked within three hours after slaughter of the animal. The other cuts were wrapped and stored at approximately 1.7°C.(35°F.±1°) for the desired time before cooking. A balanced incomplete block design, as used by Yates (1936), was employed in assigning the six treatments among the five muscle groups and in analyzing the data by analysis of variance.

Cooking: The roasts were cooked in open pans in gas ovens at 150°C. (302°F.) to an internal temperature of 63 to 66°C.(145.4 to 150.8°F.). The larger roasts from the semimembranosus, biceps femoris, and gastrocnemius, were removed from the oven at the lower internal temperature, as the temperature continued to rise after their removal. The final internal temperature of all roasts averaged 66°C. and varied from 65 to 67°C. (149 to 152.6°F.).

The weights of the raw roasts, the cooked roasts, and the pan drippings were determined. The total cooking loss, less the drippings, gave the loss by evaporation. The losses were expressed in per cent on the basis of the raw weight of each roast.

The cooked roasts were scored by four judges for tenderness, juiciness, aroma, and flavor of lean and fat. The tenderness was also determined objectively by shearing. The amount of press fluid was measured by the use of the pressometer.

Histological Studies: Slides were made of both the raw and the cooked meat. The tissues were fixed in 10 per cent formalin. Both paraffin and frozen sections were prepared. The paraffin sections were stained with French's modification of Weigert's elastic tissue stain, Harris's alum hematoxylin, and van Giesen's mixture of acid fuchsin and picric acid. From the paraffin treatment the muscle fibers were stained yellow-orange, the collagenous tissue bright red, and the elastic tissue dark blue-green. The frozen sections were stained with Harris's alum hematoxylin and Herxheimer's scarlet "R" solution, giving blue muscle tissue and red fat deposits.

RESULTS AND DISCUSSION

Effects of Storage: The external fat on the roasts cooked without storage was soft and oily. After one day of storage the fat on the other roasts was firm and brittle.

The amount of drip varied with the storage period. The roasts with zero and one day of storage were quite dry on the surface. Those with two and four days of storage were quite moist. By the ninth day the surface of the meat was again fairly dry, but the juice had collected in the folds of the wrapping paper. The roasts stored for 18 days were sticky rather than wet.

No obvious signs of growth of microorganisms were observed until the 18-day group was tested. The semitendinosus at that time showed a small

spot of blue mold. Another sign of growth of microorganisms was the sticky surface of all the 18-day roasts. This was noticed on the external fat as well as on the lean. All the 18-day roasts had rather "high" aroma when raw. This aroma persisted during and after cooking.

Rigor: With the exception of the psoas major, none of the zero-storage roasts had gone into rigor before cooking, but they were definitely in rigor when taken from the oven. The psoas major was stiff and resistant before it was cooked, but it had lost the appearance of rigor by the end of the cooking period. The extreme rigidity that made roasts in rigor difficult to cut had disappeared from the stored roasts when they were tested.

The very rapid onset and dissolution of rigor found in this study probably resulted from the extra handling the roasts received in the process of dissection and wrapping. In actual practice it is customary to ripen beef as sides, quarters, or wholesale cuts, not as individual roasts. However, to insure comparability of treatment and to prevent excessive drying of the cut surface, the roasts were cut and wrapped early in the storage period.

The roasts were extremely difficult to cut when rigor was at its peak, as they were hard and rigid and the procedure was similar to cutting a rubber cork.

TABLE 1

Average Cooking Losses and Cooking Time of Beef Roasts

Cooking losses	Time of storage (days)						F value
	0	1	2	4	9	18	
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	
Total	10.1	10.4	9.0	10.7	9.6	8.4	1.40
Dripping	1.2	1.5	1.1	1.0	0.9	3.1	4.63[1]
Evaporation	8.9	8.8	8.0	9.7	8.6	5.4	4.85[1]
Cooking time (min. per lb.)	51.9	69.9	55.0	62.1	70.4	64.3	1.26

[1] Highly significant.

Cooking Data: The data on losses and cooking time (Table 1) indicate that the total losses did not change significantly with storage. The dripping losses increased sharply for the longest storage period, but this increase was offset by a corresponding decrease in the loss by evaporation. The cooking times were too variable to show any significant change with increased storage.

Palatability Factors: The average scores are given (Table 2); no "F" value is given for flavor of fat, as many of the roasts did not have sufficient fat for judging. In addition to the palatability factors, the shear forces and the press-fluid values on the cooked beef for the different storage treatments are also given.

A decided increase in tenderness with storage was found in both the scores and the shear reading. The juiciness also increased. The aroma and flavor scores improved up to nine days but tended to decrease with further storage, owing to development of "gaminess" in the lean and rancidity in the fat. The quantity of press fluid first decreased then increased sharply, indicating changes in the water-binding powers of the proteins and the permeability of the cell membranes.

TABLE 2

Average Judging Scores, Shearing Force, and Press-Fluid Values for Beef Roasts

Palatability factors	Time of storage (days)						F value
	0	1	2	4	9	18	
Scores[1]							
Tenderness	3.5	5.8	6.6	7.0	7.9	7.7	14.58[2]
Juiciness	7.2	7.6	8.0	7.8	8.5	8.4	8.09[2]
Aroma	7.7	7.1	7.8	8.0	8.6	7.9	11.35[2]
Flavor of lean	5.8	7.4	7.8	8.2	8.6	7.9	12.27[2]
Flavor of fat	8.0	7.7	7.9	7.4	8.3	5.5	.....
Shearing force (lb.)	70.6	20.4	20.0	17.8	19.6	17.1	16.22[2]
Press fluid (pct.)	53.4	55.0	52.3	43.3	42.6	62.6	47.61[2]

[1] Maximum score was 10. [2] Highly significant.

HISTOLOGICAL CHANGES

Changes With Storage: The general microscopic changes which occurred with storage were as follows: The freshly killed beef had very poorly differentiated fibers which were straight to slightly wavy. These fibers showed the longitudinal more prominently than the cross striations. After one day of storage the fibers were much more distinct, some of them showing contracture nodes, others the kinks or crinkles of passive retraction. The nodes seemed to be very dense and resistant since they persisted through the 18 days of storage. The kinks and crinkles disappeared after four to nine days of storage. Breaks appeared on the second day and became more numerous as storage time increased. The changes during storage in the semimembranosus muscle, which are characteristic of the changes in all the muscles, are illustrated (Fig. 1).

The time of appearance of the breaks or ruptures in the fibers was variable, some sections showing them after one day of storage. No breaks were found in the zero-storage roasts.

An exception to the characteristic changes in storage was noted. After one day of storage the entire raw semitendinosus contracted, throwing the fibers into pronounced waves much longer than the crinkles produced by differences in contraction along the fibers. These waves were observed macroscopically (Fig. 2). The waves were more or less spiral, making it difficult to prepare longitudinal or cross sections (Fig. 3, lower left). When longitudinal sections could be obtained, the characteristic nodes and kinks of rigor were visible as microscopic waves within the macroscopic waves. The macroscopic waves were present after four days of storage but had disappeared by the ninth day. In this study only the semitendinosus muscle showed appreciable amounts of elastin by microscopic methods. Therefore, it is possible that the elastin content may have had a role in the formation of the macroscopic waves. The small amount of elastin in the other muscles of the round is contrary to all other observations in this laboratory. However, it should be remembered that sections of meat for microscopic study are small, hence it is possible that many samples might be obtained which showed little or no elastin.

Effect of Cooking: In general, cooking increased the microscopic manifestations of the rigor stages observed in the uncooked meat. In the zero-

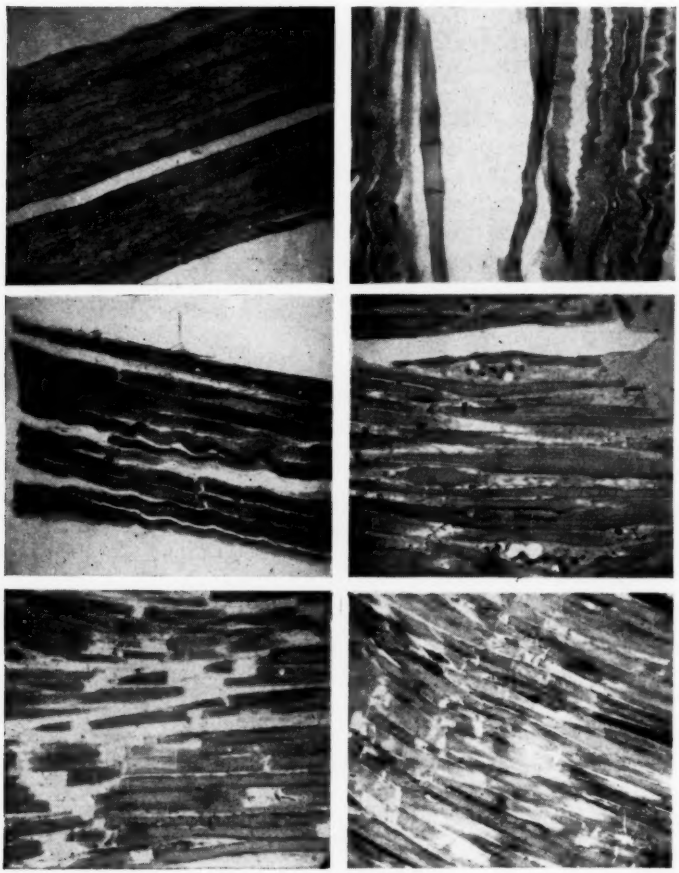

FIG. 1. Changes during storage of semimembranosus fibers (photomicrographs of longitudinal frozen sections of uncooked fibers). *Upper left:* no storage; fibers poorly differentiated and straight to wavy. *Upper right:* one day's storage; fibers show contracture nodes and crinkles of rigor. *Middle left:* two days' storage; fibers retain some waves and a few breaks are appearing. *Middle right:* four days' storage; waves have disappeared. *Lower left:* nine days' storage; breaks and disintegrated areas are more numerous. *Lower right:* 18 days' storage; similar to nine-day stored samples (40x).

storage roasts cooking increased the definition of the fibers and produced signs of heat rigor. In the one- and two-day storage roasts the indications of rigor were made more prominent by cooking. Cooking caused little change in the four-day storage roasts from that of the uncooked roasts for the same storage period. However, the roasts cooked after nine and 18 days of storage had more breaks and disintegrated areas in the fibers than the raw samples of these same roasts.

The change in the collagenous connective tissue with cooking was readily observed in the muscles having heavy deposits of this material around the muscle bundles. In the uncooked beef all the collagenous

FIG. 2. The cooked surface, after one day's storage, of the semitendinosus muscle showing the macroscopic waves of rigor (3x). (See Fig. 3, lower left, for a longitudinal section of higher magnification.)

tissue stained a bright red. However, much of this tissue in the cooked meat would no longer stain with the acid fuchsin but was colored yellow by picric acid. This cooked connective tissue was broken and somewhat granular, whereas the red-stained tissue was fibrous.

Rigor Changes: There were two distinct microscopic changes produced by normal rigor—one active, the other passive. The active change was the appearance of alternate zones of condensation and rarefaction in the fibers under tension from contracture. The passive phase was found in the crinkled or kinked appearance of the fibers, which apparently were not contracted in themselves, but were thrown in waves from outside stresses. The stresses probably came from the contraction of the noded fibers in some instances (Fig. 3, upper left) and from connective tissue in other cases.

The nodes which occurred in normal rigor were quite characteristic (Figs. 3 and 4); they were swollen and dense. The completely contracted

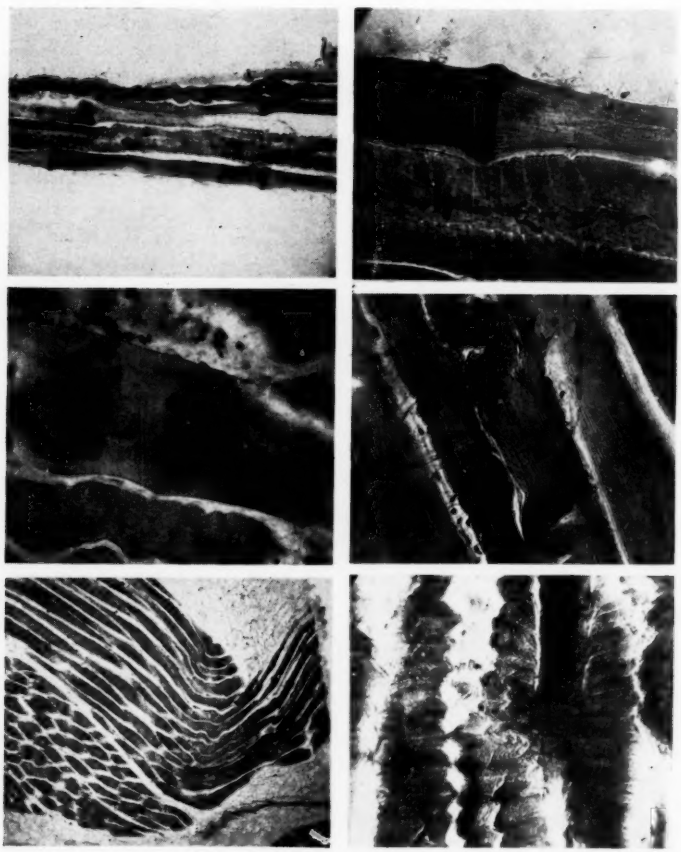

FIG. 3. Nodes, crinkles, kinks, and macroscopic waves of rigor (photomicrographs of longitudinal frozen sections). *Upper left:* cooked semimembranosus; one-day storage. Note spacing between nodes and the crinkled fibers between the noded fibers (40x). *Upper right:* same as upper left; node and adjacent crinkled fiber (180x). *Middle left:* nodes in uncooked semimembranosus after one day's storage (180x). *Middle right:* kinks in cooked vastus after nine days' storage (180x). *Lower left:* cooked semitendinosus; one-day storage; a longitudinal section of higher magnification of spiral macroscopic waves shown in Fig. 2; note kinks in bend of wave (40x). *Lower right:* crinkled raw semimembranosus fibers after one day's storage (180x).

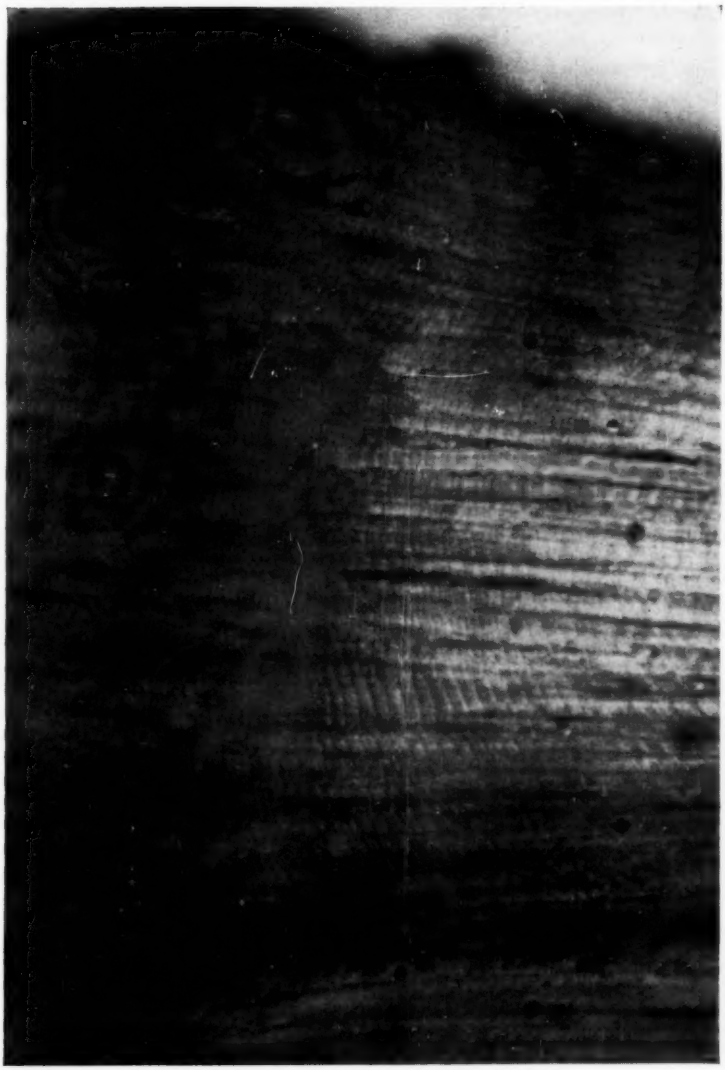

FIG. 4. Rigor node in uncooked biceps femoris, longitudinal frozen section, nine-day storage (1,870x).

nodes were too dense to show cross striations at lower magnifications. Some nodes (Fig. 3, upper right) did not show striations after magnification 1,870 times. In other nodes (Fig. 4) the condensed cross striations were visible, indicating less than maximum contraction.

The distance between the nodes varied. Sometimes two or more nodes occurred in a short space in the same fiber, whereas others were widely separated (Fig. 3, upper left and center left).

The changes in the striations around the nodes were very definite. The stretched areas immediately adjacent to the nodes in the same fibers were narrower and stained less deeply than the nodes themselves (Fig. 3, upper right). These rarified areas showed widely separated cross striations and prominent longitudinal striations, indicating stretching. The "A" discs often showed Hensen's disc, which is characteristic of fibers under tension. Close to the node the striations became very fine and much closer together.

The crinkles and kinks which occurred in the passively retracted fibers are shown (Figs. 1 and 3). The term "crinkle" is used to refer to the all-over wavy appearance of the fibers, whereas "kink" refers to the tight "S" twist seen at intervals in some of the fibers (Fig. 3, middle right). It could be seen, especially in the higher magnifications, that there was no change in the thickness or spacing of the cross striations in these passively retracted fibers, the striations merely shifting to follow the twists in the fibers.

Rigor Produced by Cooking Within Three Hours After Slaughter: Two types of nodes were produced in the muscle fibers by starting cooking of roasts before the onset of rigor. One type resembled the normal rigor nodes but was less dense (Fig. 5, upper). In the other type (Fig. 5, lower) the fibers showed irregular areas of condensation and rarefaction but the nodes and internodes were less distinct. Some fibers showed the crinkles and kinks of passive retraction, as found in normal rigor.

Breaks in Fibers: Two types of breaks appeared in the stored meat fibers—one a sharp fracture, the other a disintegration of the muscle protoplasm over a fairly long area within the fiber. The disintegrated areas had lost both the longitudinal and cross striations, the material remaining within the sarcolemma having a granular appearance (Fig. 1, lower left).

The fractures seemed to occur chiefly in the crinkled or kinked fibers. Constrictions are shown along the periphery of some of the crinkled fibers (Fig. 6, upper left). The progress of these constrictions to actual cracks in the fiber is shown (Fig. 6, upper right).

The disintegrated areas were observed most frequently in the portions of the fiber between two nodes of contracture. The narrowing of such an area is shown (Fig. 6, lower left) as well as a similar region where the rarefied part had disintegrated (Fig. 6, lower right). The extent of the granular area increased with storage, particularly at nine days and beyond.

The cause of these breaks in the fibers is not known. Two possible explanations are the autolytic action of the tissue enzymes and the physical stresses on the stretched and twisted fibers engendered by contraction of the nodes or by contraction of the connective tissue.

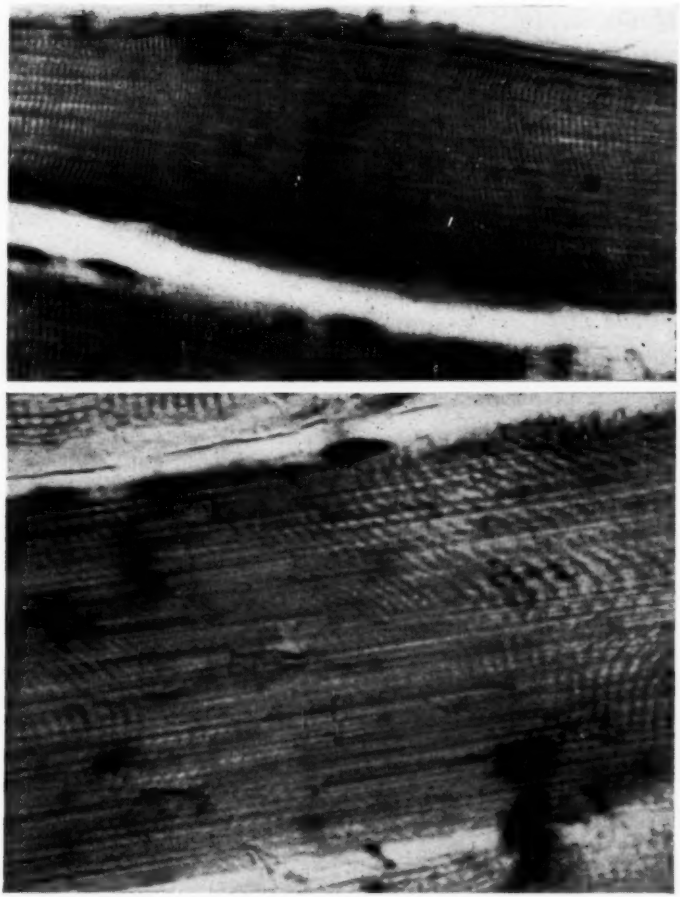

FIG. 5. Rigor nodes produced by heat in cooking (longitudinal frozen sections). *Upper:* biceps femoris; zero storage, i.e., cooked within three hours after slaughter of the animal (400x). *Lower:* less dense nodes of vastus; zero storage (800x).

CONCLUSIONS

The conclusions listed below are evaluated in terms of the definite limitation that the tests were made on only one animal. Biological variability is too great to permit any definite statements concerning an entire class, based on only one animal. Within this restriction, the conclusions possible from this study follow:

1. The findings on the changes induced by storage indicated that the greatest increase in palatability in small cuts was obtained with a nine-day storage period. Further storage led to the development of "high" or

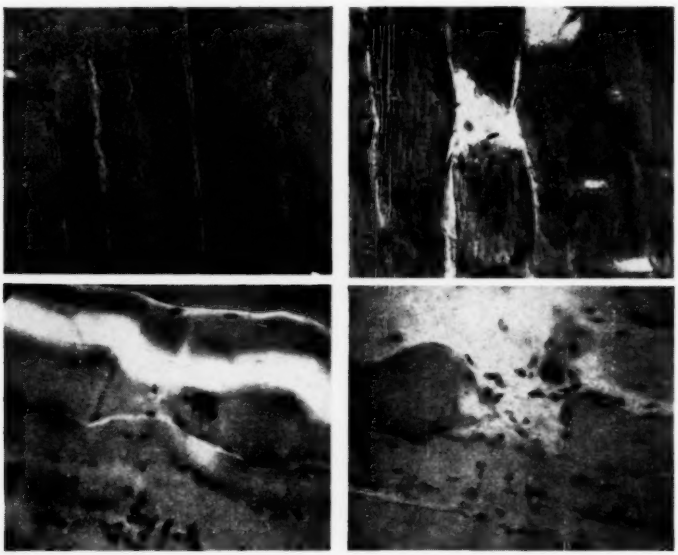

FIG. 6. The breaks and disintegrated areas appearing with dissolution of rigor (longitudinal frozen sections). *Upper left:* cooked psoas major; zero storage; some constrictions are appearing along the periphery of crinkled fibers (180x). *Upper right:* uncooked biceps femoris; nine-day storage; the constrictions have progressed to cracks (200x). *Lower left:* raw vastus; four-day storage; disintegrated area starting between two nodes (180x). *Lower right:* from raw vastus; four-day storage; disintegrated area in stretched portion of fiber between two nodes (180x).

"gamey" odors and flavors, and to rancidity of the fat. The handling of the fresh beef incident to cutting up the muscles into individual roasts before storage of the meat was considered to hasten the appearance and dissolution of rigor. Therefore, beef hung as sides or quarters would probably require a longer time in storage for the same changes to occur.

2. Rigor was shown histologically by the formation of dense nodes of contracture bordered on either side in the same fiber by areas of extreme stretch. Fibers which did not contract were drawn into waves and kinks by the shortening produced by the contracted fibers. Normal rigor produced denser nodes than those caused by heat from starting cooking of the beef before the onset of rigor.

The passing of rigor and progress of ripening were indicated by the appearance of breaks in the fibers. Sharp fractures usually occurred in the passively retracted fibers and granular or disintegrated areas in the stretched portions of the fibers adjacent to the rigor nodes.

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HEATING LAG IN THERMAL DEATH-TIME CANS AND TUBES

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In determining processes for canned foods it is necessary to know the heat resistance at various temperatures of the bacterial spores that may cause spoilage and the rate at which heat penetrates the product. The formulae developed by Ball (1928) for thermal-process calculations can be applied when the thermal death-time curve for bacteria approximates a straight line on semilogarithmic paper and the major portions of the heating curve, generally referred to as the heat-penetration curve, approximate one or two straight lines on semilogarithmic paper. The thermal death-time curve is established by a well-known technique involving a series of small containers containing spore suspensions in the material being tested which are heated for various times and at different temperatures.

Bigelow and Esty (1920), Weiss (1921), Esty and Meyer (1922), and Esty and Williams (1924) studied the thermal death time of typical thermophilic organisms and *Bacillus* (*Clostridium*) *botulinus* by techniques involving series of glass tubes containing spore suspensions. These workers used oil baths as the heating medium for the tubes and not more than 15 seconds were allowed for lag in heat penetration. Townsend *et al.* (1938) studied the thermal death time of putrefactive anaerobes, including *Clostridium botulinum*, in which they used Pyrex tubes and small metal cans. They assumed a 0.33-minute lag correction for cans based on the work of Richardson (1926), and lag corrections for tubes of 0.73 to 0.93 minute based on the work of Ball, Merrill, Williams, and Wessel (1937). These corrections are of course insignificant when the heating times are long but may be significant when the times are in the neighborhood of 10 minutes or less. Townsend (1939) measured the heat resistance of spore-forming anaerobes utilizing a water bath. He allowed a lag correction of one minute in his heating times.

Ball *et al.* (1937) measured heat penetration in tubes and arrived at the following corrections for water, sugar syrups, spinach purée, and pea purée in thermal death-time Pyrex tubes:

A. Tubes held stationary in an oil bath:

Water	3.25 min.
60% Sugar solution	3.30 min.
50% Spinach purée	4.10 min.
50% Pea purée	3.50 min.

B. Tubes agitated in an oil bath: The corrected times were approximately half of those given under A.

C. Tubes in a water bath either held stationary or agitated:

Water	0.80 min.
60% Sugar solution	1.30 min.
50% Spinach purée	1.10 min.
50% Pea purée	1.15 min.

It is the purpose of this paper to report further work on heat penetration in thermal death-time cans and tubes in order that corrections can be established more definitely for both cans and tubes. These small containers will be referred to by their conventional abbreviation, TDT cans or tubes. Terms and nomenclature used in this paper are consistent with those used by Ball (1928), Townsend, Esty, and Baselt (1938), Olson and Stevens (1939), and others.

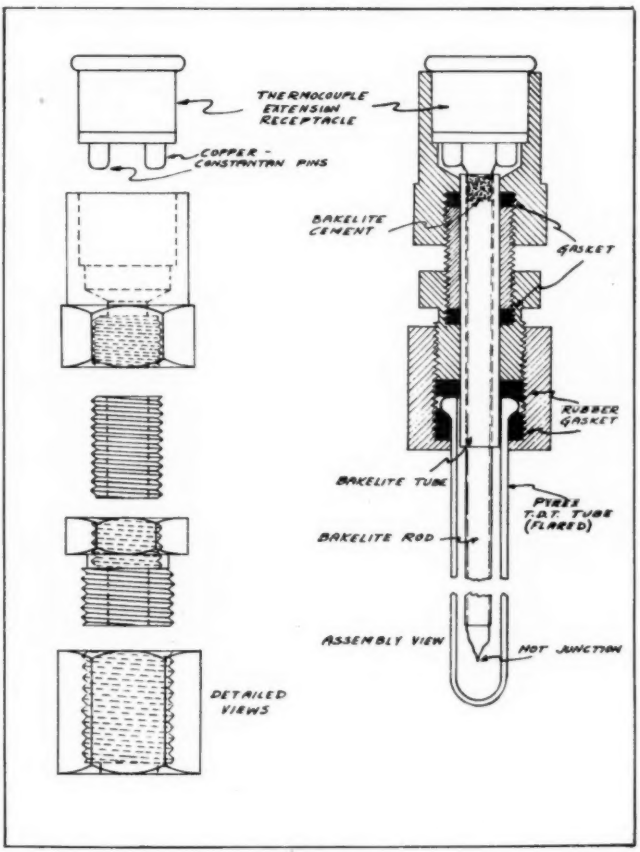

FIG. 1.

EXPERIMENTAL PROCEDURE

Heat Penetration in Pyrex TDT Tubes: The standard TDT tube used for these tests had an outside diameter of nine mm. and an inside diameter of seven mm. with an over-all length of 125 mm. The open end of the tube was flared and seated against a rubber gasket within a brass nut; a rubber gasket was also placed above the flare(Figs. 1 and 2). Into the

tube was inserted a bakelite rod (0.153 inch in diameter) which was grooved to carry the 32 B & S gauge copper and constantan thermocouple wires. The hot junction of the two wires was approximately ⅜ inch beyond the end of the bakelite rod. Near the end of the bakelite rod was drilled a hole through which a bakelite centering peg was inserted. The remaining part of this assembly consisted of suitable stuffing boxes and a thermocouple receptacle.

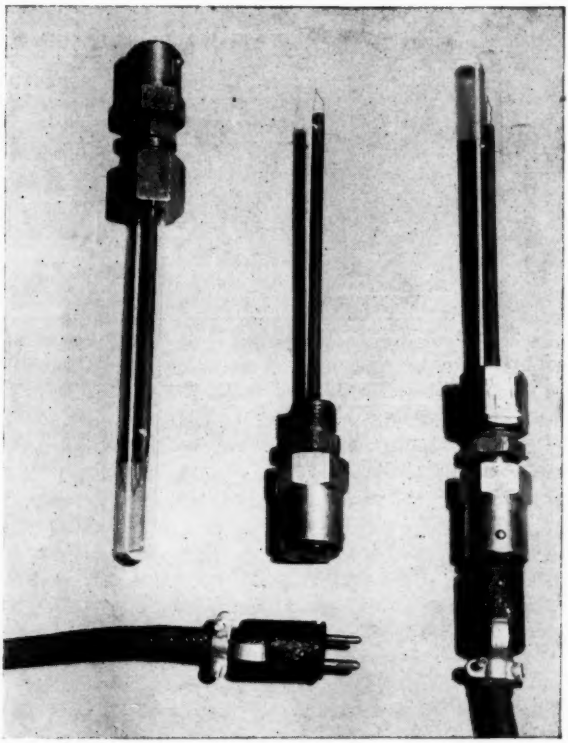

FIG. 2. Equipment for obtaining heat penetration in thermal death-time tubes.

Heat Penetration in Metal TDT Cans: The special thermocouple designed and constructed for this container and the method of assembly is illustrated (Fig. 3). A 7/16-inch hole was punched into the flat section of the bottom of the can and a 7/16-inch SAE jam nut soldered in place over this hole. The thermocouple, shown in detail in Fig. 3, was then screwed tightly to the can, using a suitable fiber washer to prevent leakage, and the wires bent in place as illustrated. The can was then ready to be filled and closed. For sealing the can it was necessary to build a special base support to accommodate the thermocouple attached to the can bottom. When this support was centered on the base plate of the

closing machine and the machine adjusted accordingly, the top could be easily attached.

It will be noted that the thermocouple hot junction is not at the geometrical can center but rather between the side and the center midway between the ends. This is because of the dimple placed in the bottom end of the can which, being so placed to aid heat penetration, moves the point of slowest heating away from the geometrical center to a point at or very near the thermocouple hot junction.

Equipment for Heating Tubes and Cans: All heat-penetration tests were made in steam at 121.1°C.(250°F.) in a small retort of about one

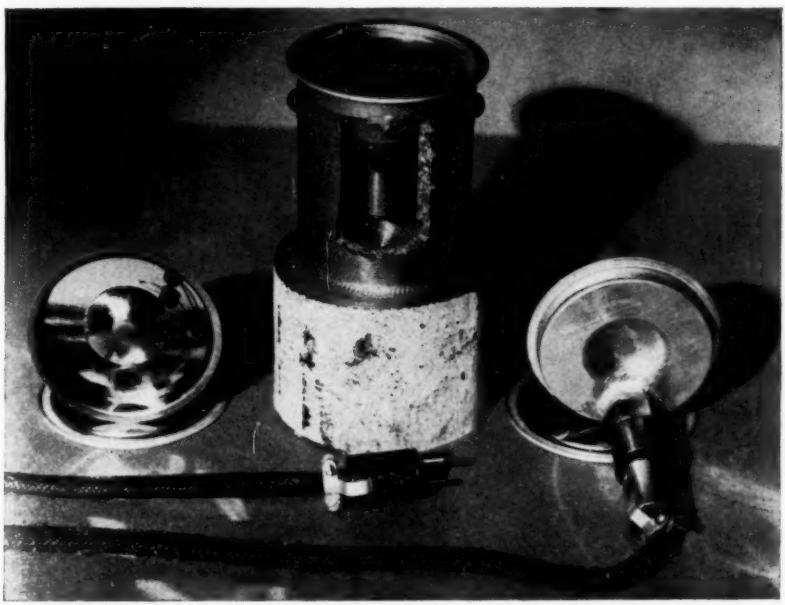

FIG. 3. Equipment for obtaining heat-penetration data in thermal death-time cans (208x006).

gallon capacity which is ordinarily used for thermal death-time studies. Steam was drawn from an ordinary three-crate vertical retort which was automatically controlled at 250°F. The small retort was equipped with a mercury thermometer, two quick-acting valves at top for exhaust and vacuum, and three quick-acting valves at the bottom for drainage, steam, and cold water. Before admitting steam, 25 inches of vacuum were drawn. When steam was admitted through the quick-acting valve, the come-up time to 250°F., by thermocouple tests, varied between 15 and 20 seconds.

Preparation of Foods: The pea purée used in these tests was prepared from canned, brine-packed peas which were comminuted in a laboratory finisher. In preparation of the purée the brine was included with the peas. Canned spinach was used without change except to cut the leaves

to fit the small can and add a proportionate amount of brine. The thermocouple hot junction was embedded between leaves of spinach. Unprocessed pork luncheon meat was obtained from a local meat packer for tests on that product.

Heating Rate Tests: Some tests were made using one ml. of material in the Pyrex tubes which resulted in approximately one inch depth of material in the tube. In other tests the depth of material was 3½ inches. The junctions of these thermocouples were located ⅝ and ⅞ inch, respectively, from the bottom of the tubes which contained 1 and 3½ inches of material. The Pyrex tubes were hermetically sealed into the thermocouple fitting for these tests.

In all cases 13 grams of material were weighed into the 2½-inch diameter and ⅜-inch high TDT cans used in these tests. These dimensions are over-all measurements. The thermocouple junction was located approximately ⅜-inch from the inside can wall along the central horizontal plane of the can. The thickness of material at this point was approximately six to seven mm. The can was sealed under 21 to 25 inches of vacuum.

The thermocouple lead wire passed through a stuffing box in the small retort and was connected to the thermocouple receptacle on the sealed tube or can. During each test, time and temperature observations were made as rapidly as possible in order that several points could be obtained for establishing the heat-penetration curve.

RESULTS AND DISCUSSION

In presenting the data only the slowest curves which were obtained in these tests are given; a summary of the data and results is given (Tables 1 and 2).

TABLE 1

Pyrex Thermal Death-Time Tubes

Run No.	Product	Heat-penetration factors		Calculated correction factor to be subtracted from experimental heating time[1]
		f_h	j	
		min.		*min.*
2-40	Water	.66	0.58	0.85
2-52	30% Sugar solution	.52	1.29	0.85
2-65	50% Sugar solution	.58	1.57	1.00
2-69	60% Sugar solution	.62	1.46	1.05
2-61	Pea purée	.72	1.42	1.20

[1] These values are for experimental heating times greater than B″ minutes—the time necessary to obtain a temperature of 0.1°F. below retort temperature—Ball (1928). They are calculated for the normal thermal death-time curve slope of 18°F.

It will be noted that the results obtained for Pyrex tubes check reasonably well with results obtained by Ball *et al.* (1937) previously quoted in this paper. The correction factors to be applied to TDT cans depend to a considerably greater extent on the particular product being tested.

It was observed that a slower rate of heat penetration was obtained when a can containing water was placed on the side or at a 45-degree angle with the horizontal than when lying flat. Since the temperature

rise in a product heating by rapid convection appears first at the top of the can and last at the bottom, the observed heating rate would be faster in a can lying perfectly flat, with the junction centered between the two horizontal surfaces of the cans, than in the can placed on its side with the junction near the bottom side. Water-correction factors were based on the side position, i.e., the position of slowest heat penetration. For a product that heats by conduction or slow convection, similar heating rates were recorded whether the can was placed on its side or lying flat during the heat-penetration tests. This was also the case with the 50- and 60-per cent sugar solution and the food products tested.

TABLE 2

Metal Thermal Death-Time Cans, 208 x 006 Conventional Dimensions

Run No.	Product	Heat-penetration factors		Calculated correction factor to be subtracted from experimental heating time[1]
		f_h	j	
		min.		*min.*
2-34	Water	0.33	1.48	0.55
2-63	50% Sugar solution	0.53	1.51	0.90
2-67	60% Sugar solution	0.78	1.00	1.20
2-38	Spinach	1.01	1.28	1.65
2-58	Pea purée	1.17	1.34	1.90
2-71	Pork luncheon meat	1.27	1.22	1.95

[1] These values are for experimental heating times greater than B″ minutes—the time necessary to obtain a temperature of 0.1°F. below retort temperature—Ball (1928). They are calculated for the normal thermal death-time curve slope of 18°F.

The slowest heating data for a can filled with 13 grams of product have been selected for the purpose of calculating correction factors for the lag in the heating of products. Any variation from this filling weight may affect the heating rates.

There is also a slight change in correction value depending upon the processing temperature (RT) employed as indicated in the following table:

TABLE 3

Effect of Processing Temperature on Correction Values for Thermal Death-Time Cans When $z = 18°F.$ and $g = 0.1$

Retort temperature	110°C. (230°F.)	115.6°C. (240°F.)	121.1°C. (250°F.)	126.7°C. (260°F.)	132.2°C. (270°F.)
Water	0.54	0.55	0.56	0.565	0.57
60% Sugar solution	1.14	1.16	1.18	1.200	1.22
Pea purée	1.87	1.89	1.92	1.950	1.98

Corrections given in Tables 1 and 2 are for RT = 250°F. to the nearest 0.05-minute time interval. However, as seen above, the variations in corrected times for RT = 230°F. to RT = 270°F. fell well within the probable error range of the corrections shown for RT = 250°F. The corrections, therefore, in Tables 1 and 2 may be applied to experimental heating times within the range of 230 to 270°F. The corrections given in

TABLE 4

Corrections for Experimental Heating Times in Steam to Give Actual TDT Times[1]

Run No.	Container	Product	Exp. heating times	Corrections to be subtracted			Corrected times		
				Z=14°F.	Z=18°F.	Z=22°F.	Z=14°F.	Z=18°F.	Z=22°F.
			min.	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
2-40	Tube	Water	1.0	0.80	0.75	0.70	0.20	0.25	0.30
			1.5	0.95	0.85	0.75	0.55	0.65	0.75
			2.0	0.95	0.85	0.75	1.05	1.15	1.25
			3.0	0.95	0.85	0.75	2.05	2.15	2.25
2-65	Tube	50% Sugar solution	1.0	0.90	0.88	0.85	0.10	0.12	0.15
			1.5	1.05	1.00	0.95	0.45	0.50	0.55
			2.0	1.05	1.00	0.95	0.95	1.00	1.05
			3.0	1.05	1.00	0.95	1.95	2.00	2.05
2-69	Tube	60% Sugar solution	1.0	0.95	0.90	0.85	0.05	0.10	0.15
			1.5	1.10	1.05	1.00	0.40	0.45	0.50
			2.0	1.10	1.05	1.00	0.90	0.95	1.00
			3.0	1.10	1.05	1.00	1.90	1.95	2.00
2-61	Tube	Pea purée	1.0	0.97	0.95	0.90	0.03	0.05	0.10
			1.5	1.20	1.15	1.10	0.30	0.35	0.40
			2.0	1.30	1.20	1.10	0.70	0.80	0.90
			3.0	1.30	1.20	1.10	1.70	1.80	1.90
2-34	Can	Water	0.5	0.48	0.46	0.45	0.02	0.04	0.05
			1.0	0.60	0.55	0.50	0.40	0.45	0.50
			1.5	0.60	0.55	0.50	0.90	0.95	1.00
			2.0	0.60	0.55	0.50	1.40	1.45	1.50
2-63	Can	50% Sugar solution	1.0	0.90	0.85	0.80	0.10	0.15	0.20
			1.5	0.95	0.90	0.80	0.55	0.60	0.70
			2.0	1.00	0.90	0.80	1.00	1.10	1.20
			3.0	1.00	0.90	0.80	2.00	2.10	2.20
2-67	Can	60% Sugar solution	1.5	1.20	1.15	1.05	0.30	0.35	0.45
			2.0	1.30	1.20	1.10	0.70	0.80	0.90
			3.0	1.30	1.20	1.10	1.70	1.80	1.90
2-38	Can	Spinach purée	1.5	1.40	1.35	1.30	0.10	0.15	0.20
			2.0	1.65	1.55	1.45	0.35	0.45	0.55
			3.0	1.75	1.65	1.55	1.25	1.35	1.45
			4.0	1.75	1.65	1.55	2.25	1.35	2.45
2-58	Can	Pea purée	2.0	1.80	1.70	1.60	0.20	0.30	0.40
			2.5	1.95	1.85	1.75	0.55	0.65	0.75
			3.0	2.00	1.90	1.80	1.00	1.10	1.20
			4.0	2.10	1.90	1.80	1.90	2.10	2.20
2-71	Can	Pork luncheon meat	2.0	1.80	1.70	1.65	0.20	0.30	0.35
			3.0	2.10	1.95	1.85	0.90	1.05	1.15
			4.0	2.10	1.95	1.85	1.90	2.05	2.15
			5.0	2.10	1.95	1.85	2.90	3.05	3.15

[1] For experimental heating times greater than those shown the correction factor is obviously the same as that shown for the maximum time.

Table 3 for retort temperatures other than 250°F. were calculated by the same method, Ball (1928), using the heat-penetration data shown in Table 2 applied to the different retort temperatures.

The length of experimental heating time (B_R) may be shorter than the heating time (B″) required to heat the product to a temperature 0.1°F. below retort temperature. If so, the corrections given in Tables 1 and 2 do not apply and reference should be made to Table 4.

TABLE 5

Results of Thermal Death-Time Tests on Pea Purée Packed in TDT Cans

Process temperature	Experimental heating times		Corrected heating times	
	Survival	Destruction	Survival	Destruction
°F.	*min.*	*min.*	*min.*	*min.*
230	50.0	80.0	48.1	78.1
240	19.0	25.0	17.1	23.1
250	4.5	6.0	2.6	4.1
260	2.6	2.9	0.7	1.0

The correction factors also vary with different values of the thermal death-time curve slope (Z). In Table 4 are given correction factors for various heating times and z values of 14 to 22°F. The great majority of thermal death-time slopes fall within the range of 14 to 22°F.

The corrected heating times given in Table 4 were obtained by subtracting from the experimental heating time the lethal value of the process, heating and cooling portions combined, expressed as minutes at retort temperature. These lethal values were determined by the method of Ball (1928). Because the experimental heating times included the 15 to 20 seconds required to bring the retort to temperature, the indicated correction factors include both the retort lag and the heat-penetration lag.

From the results given (Table 4) it appears that the heating rate for products that heat by convection (water) is slightly faster in the can than in the tube. However, as heat transfer changes from a convection to a conduction basis the heating rate in the tube becomes faster than in the can. To illustrate the application of these factors, data from a sample thermal death-time test are taken as shown (Table 5).

The microorganism used in these resistance tests was the Cameron organism No. 3679, a putrefactive, gas-producing anaerobe classified as *Clostridium sporogenes*. The concentration per 208 x 006 can was between 150,000 and 200,000 spores. The pH of product was 5.5.

When plotting these data on semilogarithmic paper the curves given (Fig. 4) were obtained. It will be observed that a straight line on this paper fits the corrected points much better than the uncorrected ones. It is also apparent that the thermal death-time curve characteristics are quite different for the "best fit" straight lines illustrated. This is shown by the following comparison:

	Corrected	Uncorrected
F	4	7.8
Z	16	22.0

The error introduced by failure to apply the proper correction factor will, of course, vary with the magnitude of the thermal death time. With relatively long times the error would be insignificant.

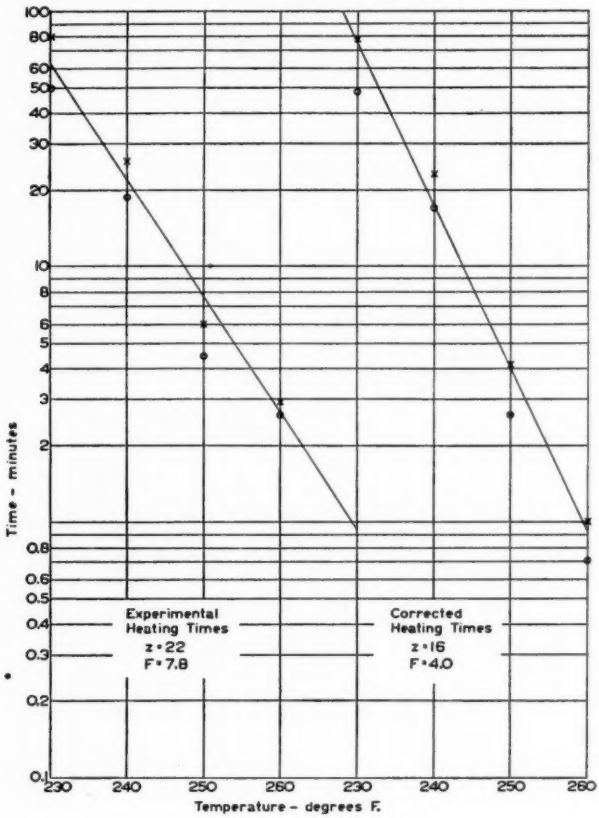

FIG. 4.

SUMMARY

It has been demonstrated that when making thermal death-time tests involving relatively short times, the heat-penetration lag and the retort come-up time take up an appreciable percentage of the total thermal death time. Correction factors for these lags have been determined for a number of products. When the factors are used in correction of come-up and heat-penetration lags in thermal death-time studies, lower F and z values are obtained than when instantaneous heating and cooling is assumed without applying the corrections.

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AMINO ACIDS AND RELATED COMPOUNDS IN HONEY[1,2]

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Honey, by official definition, is the nectar and saccharine exudation of plants gathered, modified, and stored in the comb by the honeybee; by chemical definition it is a natural, unrefined, concentrated syrup of invert sugar having a flavor which is characteristic of its floral source. Among its minor components there are, besides mineral matter, organic acids, pigments, ascorbic acid, and some seven members of the B-vitamin complex, nitrogen-containing compounds. Some of the latter are proteins; others are of an amino-acid character reactive with triketohydrindene hydrate (ninhydrin).

Paine, Gertler, and Lothrop (1934) have pointed out that some of these foreign substances are in the colloidal state and that they affect certain of its properties which are important in the technology of honey production and utilization. Frothing, scum-formation, and a caramelization when honey is heated are its undesirable characteristics with respect to utilization; lack of clarity and brilliance of appearance in competition with other syrups for the retail trade are pertinent to its production although perhaps more significant before the days of sugar rationing than now.

Lothrop and Gertler (1933) observed that the colloids isolated from honey will decompose to an appreciable extent when subjected to temperatures above 50°C.(122°F.) and that even though the honey itself be treated so as to remove practically all of these constituents, it will still be more subject to caramelization on heating than is a mixture of dextrose and levulose of approximately the same concentration. Amino acids and probably also other products of protein hydrolysis, although present in honey to the extent of only several thousandths per cent, are suspected of being responsible, in part if not wholly, for this phenomenon. The possibility of melanoidin formation in the interaction of its reducing sugars and amino acids is not mere speculation. Little or no evidence exists to show that melanoids are present in fresh honey.

That the amino-acid content of a honey is affected by time and processing and storage temperatures has been found by Lynn, Englis, and Milum (1936) to be true with respect to a sample of sweet clover honey. This conclusion is not necessarily pertinent, however, to honeys of all the color grades in which this product appears on the market.

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[2] Constructed from a thesis submitted by Clayton L. Baldwin, Jr., to the faculty of the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Master of Arts, June, 1939.

An interest in the existence of a probable relationship between degree of pigmentation of honey and the content of its minor, nonsugar constituents having been an object of research in this laboratory for some years, it seemed worth while to include in such a study the amino acids. Added interest in this survey lay in the fact that Lothrop and Gertler (1933), examining 10 honeys from which the colloids had been removed, had observed that "in a general way" a correlation existed between the amount of amino nitrogen in the honey, determined with the aid of the nynhydrin reaction as adapted by them to the peculiar conditions existing therein, and its tendency to caramelize on heating.

There were at hand 37 representatives of the whole gamut of color classification which is used in the merchandising of honey—Sechrist (1925)—from the so-called water-white to dark. With few exceptions, and these were bottled blends for the retail trade, they had been obtained from the growers themselves in Arkansas, California, Florida, Georgia, Illinois, Minnesota, New York, Ohio, Texas, Virginia, West Virginia, Wisconsin, and the Hawaiian Islands. Included herein were honeys whose known predominant floral sources were, respectively, alfalfa, algarroba, blue-curls, buckwheat, several varieties of clover, cotton blossom, gallberry, holly blossom, horsemint, mesquite, orange blossom, pepper bush, sage, spikeweed, tamarisk, and wild flowers.

EXPERIMENTAL PROCEDURE

In the light of the experience of others the choice of analytical methods appeared to lie on the one hand in the gasometric, using a modified micro-Van Slyke apparatus as did Lynn *et al.* (1936), and, on the other, in the colorimetric. The latter traces its origin to the researches of Ruhemann (1910 a, b) on cyclic triketones, its appraisal as a sensitive reaction to the investigations of Riffart (1922), and its adaptation to sugar products to Ambler (1927) and to honey, specifically, to Lothrop and Gertler (1933). Our preference was for the nynhydrin procedure, using bentonite—Lothrop and Paine (1931)—to remove proteins, Neuberg and Kerb (1912) reagent to precipitate the amino acids, and hydrogen sulfide to regenerate them as a prelude to the formation of the characteristic violet-colored complex, its purification in turn being based on the suggestions of Polonovski and Moreno-Martin (1935). In general, with some modification, the experimental technique of Lothrop and Gertler (1933) was used.

To a 60-gram sample of honey in a 250-ml. Erlenmeyer flask there are added seven ml. of a five-per cent suspension of bentonite and 43 ml. of water. The flask is stoppered, shaken vigorously, allowed to stand overnight to insure complete flocculation, and then filtered into a wide-mouthed flask, due precautions being taken to prevent evaporation losses.

Exactly 40 gm. of this protein-free honey solution, containing 21.8 gm. of the original honey, are weighed into a 400-ml. beaker and then mixed with 200 ml. of 95-per cent alcohol. While the whole is being stirred, 1 *N* sodium carbonate solution is added from buret until the system becomes slightly basic toward bromthymol blue solution used on a spot plate. Then, alternately, one-ml. portions of freshly prepared 1 *N* mercuric

acetate solution and the same sodium carbonate solution are added until the mixture has assumed a distinctly yellow or orange color, a slightly alkaline reaction being desirable. Beaker and contents are then let stand overnight, after which the supernatant liquid is poured off and the precipitated mercury compounds transferred to a tube for centrifugal separation and washing with 80-per cent alcohol solution, the supernatant liquid being discarded after each treatment. Four or five 20-ml. portions of alcohol are usually sufficient for the removal of practically all the sugars.

The washed precipitate is then suspended in a test tube in 40 to 50 ml. of water to which four to five drops of concentrated hydrochloric acid are added. The tube is immersed in a boiling-water bath, and hydrogen sulfide vigorously passed in for 10 minutes, the tube being occasionally shaken in order to keep its contents in suspension. After the precipitated sulfides have settled, the contents of the tube are filtered, precipitate and filter paper being washed with dilute hydrochloric acid solution. Filtrate and washings are evaporated in a beaker to a volume of about 15 ml., two drops of phenolphthalein solution are added, and then dilute sodium hydroxide solution is added dropwise until the whole is faintly alkaline. Finally, very dilute hydrochloric acid is added just to the disappearance of the color. The solution, after having been made up to a volume of 25 ml., is ready for the nynhydrin test.

In carrying out this reaction one ml. of the above solution, two ml. of a freshly mixed buffer solution consisting of three parts $M/15$ Na_2HPO_4 and two parts $M/15$ KH_2PO_4, and one ml. of nynhydrin test solution (one per cent) are mixed in a test tube. One-ml. portions of the standards are treated likewise. All are heated in a constant-level, boiling-water bath for one-half hour, removed, and allowed to cool. The contents are quantitatively transferred to a 100-ml. volumetric flask, the total volume of water used being approximately 70 ml. To this solution 10 ml. of chloroform are added. The whole is then acidified by the addition of 10 to 15 drops of glacial acetic acid and vigorously shaken. The colored chloroform layer should be protected as much as possible from light because of its instability. The aqueous layer is poured off, 75 ml. of fresh water containing one drop of glacial acetic acid per 10 ml. added, and the whole shaken. The chloroform solution of the violet-colored complex is washed thus three times. Finally it is shaken with 70 to 80 ml. of 0.01 N sodium hydroxide solution whereupon the color returns to the aqueous layer. The solution is then made up to the mark and compared in a colorimeter with a standard similarly treated, which is prepared from a stock solution of aspartic acid containing 100 mg. of nitrogen per liter.

In carrying out the analyses in question, standards were prepared by adding three, five, eight, and 10 ml. of the stock aspartic acid solution to 15-ml. portions of a 70-per cent invert sugar solution. Alcohol was added to each standard, after which it was given the same mercury precipitation and subsequent treatment as the unknown solution. The final violet coloration resulting from the treatment with the 0.01 N sodium hydroxide solution is quite stable. It was not found necessary to make new standards for every series of analyses.

TABLE 1

Amino-Nitrogen Content of Light Honeys

Predominant floral source	Origin	Color grade		Amino-nitrogen content
		Pfund scale	Description	
				pct.
Algarroba	Hawaii	0.3	Water-white	.0014
Sweet clover	Wisconsin	0.6	Water-white	.0069
White sage	California	0.7	Water-white	.0008
Clover	Ohio	1.3	Extra white	.0098
Orange blossom	California	1.5	Extra white	.0015
White clover	Wisconsin	1.8	White	.0017
White clover	Wisconsin	1.8	White	.0024
Clover	West Virginia	2.4	White	.0056
Mesquite	California	2.7	White	.0041
Gallberry	Georgia	2.9	White	.0019
Clover	Wisconsin	3.8	White	.0075
Unknown	Illinois	3.9	Extra light amber	.0021
Blue-curls	California	4.0	Extra light amber	.0024
Wild flowers	Virginia	4.0	Extra light amber	.0021
Tupelo	Florida	4.5	Extra light amber	.0009
Horsemint	Texas	4.7	Extra light amber	.0032
Average	.....	.....	.....	.0034

TABLE 2

Amino-Nitrogen Content of Dark Honeys

Predominant floral source	Origin	Color		Amino-nitrogen content
		Pfund scale	Description	
				pct.
Buckwheat	Minnesota	4.9	Light amber	.0021
Tupelo	Florida	5.0	Light amber	.0026
Thistle	California	5.5	Light amber	.0030
Cotton blossom	Texas	5.7	Light amber	.0020
Sage, buckwheat	California	5.8	Light amber	.0013
Unknown	Illinois	5.8	Light amber	.0028
Unknown	Illinois	5.8	Light amber	.0011
Holly blossom	Arkansas	6.0	Light amber	.0044
Alfalfa	California	6.4	Light amber	.0042
Cotton blossom	Georgia	6.6	Light amber	.0061
Alfalfa	California	6.6	Light amber	.0053
Tupelo	Georgia	6.7	Light amber	.0039
Mexican clover	Georgia	7.8	Light amber	.0022
Alfalfa, sweet clover	California	8.0	Light amber	.0056
Pepper bush	Georgia	8.2	Light amber	.0009
Tamarisk	California	8.7	Amber	.0020
Buckwheat	Wisconsin	8.7	Amber	.0034
Spikeweed	California	9.0	Amber	.0059
Unknown	Georgia	10.5	Amber	.0061
Buckwheat	Wisconsin	11.3	Amber	.0375
Buckwheat	New York	14.2	Dark	.0200
Average	.....	.....	.....	.0058

RESULTS AND DISCUSSION

Data for each of the samples analyzed (Tables 1 and 2) during the course of this survey have been made a part of this record, although primarily the center of interest was the degree of pigmentation rather than the floral source of the honey. This emphasis is consistent with current merchandising practices, for no requirement in this respect is made in the United States for grades of honey except that covered by the phrase "freedom from damage caused by objectionable flavor." According to its position on the Pfund scale—Sechrist (1925)—each honey was assigned to a "light" or "dark" classification as typifying, in a qualitative sense, the most obvious differences in pigmentation. The effect of this segregation was assignment of those grading water-white, extra white, white, and extra light amber to the first group; light amber, amber, and dark to the other (Table 3).

TABLE 3

Amino-Nitrogen Content of Light and Dark Honeys

Classification	Samples	Amino-nitrogen content
Light honeys		*pct.*
Water-white	3	.0030
Extra white	2	.0057
White	6	.0038
Extra light amber	5	.0021
Average	....	.0034
Dark honeys		
Light amber	15	.0031
Amber	5	.0110
Dark	1	.0200
Average	....	.0058

In interpreting the values found for nitrogen owing to amino acids and related compounds by the nynhydrin test, it should be borne in mind that only a portion of the noncolloidal, nitrogenous constituents of honey are in a form simple enough to give the characteristic reaction. The formation of the violet-colored complex depends on the group $R \cdot CH \cdot NH_2 \cdot COOH$, but substances more complex than this produce a coloration also. Fortunately, the proportion of nitrogen-containing compounds, other than the simple amino acids in honey, is deemed to be very small; hence the error, if any, from this source is not large. Finally, the exact tint of the color developed varies, depending on the particular amino acids present. However, after the chloroform-purification treatment it was found possible to match very well all "unknowns" with the aspartic acid-containing standards. The data have informative, qualitative values.

SUMMARY

Amino acids and other ninhydrin-reactive compounds are minor components of honey. In the analysis of 16 light and 21 dark samples from three crops, representing approximately 20 floral types and all the com-

mercial color grades, the following values were found: maximum 0.0375 per cent; minimum 0.0008 per cent; average, 0.00476 per cent. Degree of pigmentation is apparently a factor here, for the dark honeys were found to contain more of these compounds than did the light ones.

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A STUDY OF THE BACTERIAL FLORA OF MACKEREL[1]

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The preservation of fresh fish by fast-freezing is an industry which is undergoing logical development because of certain advantages which it affords. It offers speed and economy, and the characteristics of content of flavor and vitamin are retained. It was decided to investigate the effect of fast-freezing upon the bacterial flora of mackerel, which is one of the important fish for food of the California coast and which is available regularly. Numerous examinations of the flora of fish from marine waters have been made previously and they have yielded comparable results; nevertheless, in a problem of this nature it was necessary to acquire data regarding the nature and amount of the bacterial flora of this particular variety of fish in this locality in order to determine the effect of freezing upon its microorganic content.

WORK OF PREVIOUS INVESTIGATORS

Hunter (1920b) determined that the flora of decomposing salmon was the same as that of sea water and described it as consisting of soil, water, and sewage microorganisms. Reed and Spence (1929), in their study of intestinal and slime flora of haddock, reported the presence of *Proteus, Pseudomonas, Achromobacter, Flavobacterium,* and *Bacillus* in both intestinal contents and slime. *Micrococcus* was reported chiefly from slime, while colon forms—*Escherichia, Salmonella, Aerobacter,* and *Eberthella*—were demonstrated only in the intestine.

Sanborn (1930) studied the bacterial decomposition of fresh, smoked, and iced fish and reported that marine bacteria were constantly in or on fresh fish and on smoked and frozen fish. They were active even at low temperatures; most of them were proteolytic and a few were putrefactive. Smoked fish were attacked by *Torula, Penicillium,* and *Achromobacter,* while the iced fillets were invaded by *Proteus, Eberthella, Micrococcus,* and *Cheatostylum,* all strains of which withstood —18 to —23° C.(—0.4 to —9.4°F.) for months. Bedford (1933a), in his survey relating to preservation of fish, reported the following genera of bacteria to be present: *Achromobacter, Flavobacterium, Serratia, Micrococcus,* and *Rhodococcus.* The quantities on the surface of the fish were smaller than those found in the sea in the vicinty, while the numbers in the alimentary canal of a fish depended upon its feeding habits; bottom feeders contained larger numbers than did others. Wood (1939) reported the isolation of *Micrococcus, Flavobacterium, Achromobacter, Pseudomonas,* and *Bacillus* from fish

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taken from eastern Australian waters. The flora of the slime and gut was the same as that of the spoiling muscle. The bacteria of the external environment between the time of catch and consumption were identical with those of the fish. Wood established a generic succession in the flora of spoilage.

In a comprehensive survey of the bacterial flora of North Pacific salmon Snow and Beard (1939) were able to classify into 15 genera 1,838 strains of microorganisms isolated. (Yeasts were not considered.) These included *Achromobacter, Micrococcus, Pseudomonas, Kurthia, Flavobacterium,* and *Proteus,* which appeared consistently from all sources, as well as *Sarcina, Rhodococcus, Lactobacillus, Bacillus, Serratia, Staphylococcus, Streptococcus, Aerobacter,* and *Escherichia,* which were encountered less frequently. No evidence of the presence of obligate anaerobes was obtained.

The problem as to whether fish have a typical intestinal flora has been given attention. As early as 1904 Johnson performed experiments which led him to conclude that *Escherichia coli commune* was not a natural inhabitant of the intestine of fish but that it was taken up during feeding. Browne (1917) determined the presence of *E. coli* and *Clostridium welchii* in scup from unpolluted waters but it is to be remembered that scup are bottom feeders. Hunter in 1920 failed to isolate *E. coli* from salmon but Gibbons (1934) found lactose fermenters in nearly all off-shore mackerel. It is generally considered, however, that *E. coli* does not occur normally in the intestines of fish other than in those which are in or which have been feeding in polluted water.

Early workers believed that the flesh of freshly caught, live fish was sterile. Hunter (1920a), in a study of decomposition of salmon, reported that the muscles of freshly taken fish were free of microorganisms but that subsequent infection took place rapidly. He also pointed out that washed fish decomposed more slowly than did those unwashed. Moreover, work showed that the digestive tract of the fasting salmon, i.e., salmon on the way to its spawning grounds, was sterile and he cited the research of Obst (1919) with sardines which yielded a similar outcome. He suggested that since the blood did not coagulate completely, the various organs of a fish were infected through the blood stream within 96 hours after its death.

Procter and Nickerson (1935) studied the muscle of haddock frozen solid by use of carbon dioxide within one hour of removal from water. Cultures from the muscle and others prepared from the blood of the live fish were negative. The work of Gee (1927) was not in agreement with these authors inasmuch as he reported the recovery of viable bacteria from the muscles of dog fish and cod but he found none in other species examined inclusive of flounder, alewives, mackerel, silver and squirrel hake, and pollock. Trap netting furnished a higher proportion of sterile fish than did other methods of capture.

The bacterial discoloration of fish has been studied by Bedford (1933b), who was able to produce the characteristic alteration experimentally in halibut by use of *Pseudomonas fluorescens.* The organisms grew at a temperature as low as —5°C.(23°F.). Harrison and Sadler (1929) had shown previously that the organisms were not on the fish when caught nor were

they demonstrable in the sea, but they were present in artificial ice and in the bilges and holds of ships.

The bacterial count in muscles of edible fish may be high. Stansby and Griffiths (1934) stated that counts of more than 10^6 organisms per gram of fish indicate that spoilage will be rapid and they decided that fish with more than 10^7 bacteria per gram were not marketable.

EXPERIMENTAL PROCEDURE

Qualitative: The fish to be examined were brought to the laboratory from the local markets or directly from the fishing boats and were packed in ice until time for testing. They were weighed, measured, and examined for external signs of decomposition. Then the skin over the back muscles, posterior to the pectoral fin, was seared with a hot spatula and a sterile wire loop was passed into underlying tissue. The sample was streaked to a fish infusion agar plate. This fish infusion made from whole mackerel included 1.5 per cent of sodium chloride and its final pH was 7.4 to 7.6.

After making preparations from the muscle, the fish was opened aseptically and the liver and heart blood were cultured. The body was then examined for internal signs of spoilage and for parasites. The digestive system was opened and samples of content of intestine and stomach were likewise streaked to the fish infusion agar plates. The reason for this method of inoculation was twofold: first, it was determined that the bacterial content of the intestine was sufficiently small so that one plate only was required, and second, it was observed that more diverse and copious growth appeared on streaked plates than upon those which were prepared by inoculating the organisms into melted, cooled agar. Even though the agar was cooled to 40°C.(104°F.) before seeding and pouring, growth was less than that obtained by streaking. This finding is in agreement with the work of ZoBell and Conn (1940) on the thermal sensitivity of marine bacteria in which these authors showed that only 20 per cent of marine organisms in sea water or mud would withstand heating to 40°C. for 10 minutes. The cultures were all incubated at room temperature for a minimum of four days.

When good growth had developed on the plates, it was studied; representative colonies were picked, they were purified by restreaking and then were transferred to fish infusion agar slants.

Ordinary differential media, without added salt, were used, since it was found that most of the cultures would multiply on them. The resulting growth often was not very heavy and this was especially true in the case of the tryptone broth which was utilized to test for production of indol. The formulae employed for examination of the cultures were the following: glucose fermentation tubes, nitrate broth, litmus milk, lead acetate agar, and gelatin. In addition to these, certain ones of the cultures were placed in contact with lactose, maltose, sucrose, xylose, salicin, and mannitol but these carbohydrates were not routinely used inasmuch as the organisms concerned in many cases do not ferment them. The cultures in differential media were incubated at room temperature for six days before examination but those in litmus milk, gelatin, and lead acetate were incubated for at least 12 days before they were discarded because changes

in these preparations were observed frequently to appear later than the sixth day.

All cultures which had been isolated from mackerel were inoculated later into sea water-tryptone broth and incubated for seven days at room temperature. They were then tested for indol by the method of Ehrlich. Control cultures known to produce indol were tested at the same time in the same batch of medium, but although the controls yielded positive tests for indol, none of the cultures from the mackerel produced this cleavage product. Wood (1939) also commented on the infrequency of indol-producing microorganisms in fish, while Hunter (1920a,b), on the other hand, used only indol producers or organisms producing a foul odor in fish broth in his studies on the decomposition of salmon. Snow and Beard (1939) reported the formation of indol by many of the cultures isolated by them. C. E. ZoBell (personal communication), however, stated that only two of more than 70 cultures of marine bacteria characterized in his laboratory formed this by-product.

No attempt at identification of species was made, but on the basis of the nature of the activity appearing on the differential media, the gram stains, morphology of colony, and determination of motility the following genera were identified: *Micrococcus, Sarcina, Streptococcus, Pseudomonas, Flavobacterium, Lactobacillus, Kurthia,* and *Escherichia.* The remainder of the group would probably fall into the heterogeneous family, Bacteriaceae, Bergey (1939). These last were mainly *Achromobacter.* The *Achromobacter* were those most frequently present but the *Micrococci* were demonstrable in numbers nearly as large. It is a little surprising, in view of the statements of other workers, that *Pseudomonas* and *Flavobacterium* appeared more rarely.

In addition to the above, two strains of *Actinomyces* and one *Aspergillus* were isolated. They were, however, representatives of colonies which did not occur regularly on the plates. Only two strains of agar-digesting bacteria were recognized.

Anaerobic cultures of the intestinal contents of 12 fish were set up in fish fusion agar by use of the Spray technic (1930) but no obligate anaerobes were demonstrated. This is in agreement with the observations of Hunter (1922) and of Snow and Beard (1939) who likewise failed to demonstrate obligate anaerobes.

The absence of sporeformers is notable. This finding is in direct contrast to the work of Gee (1927) and of Reed and Spence (1929). Snow and Beard (1939) also reported *Bacilli* as being common although not among the six genera most usually encountered.

The cultures from the stomach and intestinal contents all gave positive growth but the numbers varied from a few colonies to many. Of the other attempts to culture, 20.6 per cent of those made from the back muscles, 26.5 per cent of those from liver, and 41.2 per cent of the preparations from the heart blood showed growth. This suggests that contamination of the tissues of the fish may come about through the blood stream, either at the time of death or soon thereafter, and is in accord with that opinion by Hunter (1920a,b).

Quantitative: The technique of sampling the muscle of the fish for quantitative examination was as follows: The skin posterior to the pectoral fin was seared and removed aseptically. A sample of the exposed tissue beneath, weighing between three and four grams, was removed and placed in a sterile, tared bottle with glass beads. The bottle and sample were weighed and sterile sea water added to equal a dilution of 1 to 10. The muscle was then fragmented by means of vigorous shaking. Duplicate dilutions of 10^{-2} and 10^{-3} were made in sterile sea water and plated in triplicate upon fish infusion agar. The intestines were sampled by squeezing some of the contents into a sterile, tared bottle, after which the procedure was the same as that utilized for the muscle. All plates were incubated for six days at 21 to 25°C.(69.8 to 77°F.) before counting.

TABLE 1

Bacterial Content of Muscle and of Intestinal Contents of Mackerel

Fish No.	Bacteria per gram of muscle	Bacteria per gram of intestinal contents
1	70	60,000,000
2	15,800	64,000,000
3	None	.....
4	None	11,000
5	None	3,700,000
6	3,600	5,000,000
7	50	.....
8	130	10,000
9	30,000	.....
10	320	10,000
11	390	.....
12	None	1,500
13	650	70
14	7,350	7,000
15	160	3,900
16	40[1]	.....
17	1,285	.....
18	1,940	.....
19	50[1]	.....
20	235	.....
21	175	.....
22	540	.....

[1] Based on counts of less than 30 colonies.

The results of examinations of the muscle of the fish (Table 1) are the averages of plates which developed from 30 to 300 colonies each unless designated otherwise. It is demonstrated that usually the flesh of fish was not sterile although its count was well within the limit set by Birdseye (1929), Griffiths and Stansby (1934), and Griffiths (1937) for marketable material. It is to be noted that the content seldom exceeds a thousand bacteria per gram of back muscle. Kiser and Beckwith (1942) showed, however, that in similar fish the count in the muscle of the flank might be as high as 5,000 per gram. This is in agreement with the work of Birdseye (1929), who has stated that the count of the back muscle of

some fish may be less than 100 per gram but the same fish may contain as many as 10^6 bacteria per gram in its flanks. The bacterial content of the intestine ranged from less than 100 to more than 10^7 bacteria per gram.

A second series was prepared in which the dilutions were plated on fish infusion agar and on a formula suggested by ZoBell (1941), which is a sea-water medium containing no added source of growth stimulants, such as beef or yeast extract. The number of fish included in the results of the second series (Table 2) is small but it is definitely indicated that

TABLE 2

Comparison of Media Used for Isolation

Fish No.	Bacteria per gram of muscle		Bacteria per gram of intestinal contents	
	ZoBell's	Fish infusion	ZoBell's	Fish infusion
1	20	10	.....	.....
2	70	0	.....	.....
3	40	0	.....	.....
4	25	0	.....	.....
5	15,800	3,000	300,000	300,000
6	None	None	1,000,000	300,000

ZoBell's medium is superior to the fish infusion agar for isolating the organisms from fish.

SUMMARY

The bacterial flora of 34 mackerel was examined qualitatively. The heart, liver, muscles of the back, and contents of stomach and intestine were sampled. The cultures derived showed that the organisms preponderantly present were *Micrococcus, Achromobacter, Pseudomonas, Flavobacterium, Sarcina, Kurthia, Lactobacillus,* and *Streptococcus.* One strain of *Escherichia* was encountered.

Quantitative examination of back muscle and of intestinal contents showed fewer than a thousand bacteria per gram of muscle in most instances but the intestinal microorganic content was occasionally of the order of 10^7 per gram.

It was shown that a relatively simple formula based on the use of sea water was superior to fish infusion agar as a medium for the quantitative bacteriological examination of fish.

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EFFECTS OF TEMPERATURES APPROXIMATING 0°C. UPON GROWTH AND BIOCHEMICAL ACTIVITIES OF BACTERIA ISOLATED FROM MACKEREL[1]

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The work herein described was undertaken in connection with a study of the effect of fast-freezing upon the bacterial flora of mackerel. The purpose of the experiments was to ascertain the effects of the low temperatures used in the process upon the characteristics of growth of these bacteria which represent a major portion of the bacterial flora of the fish.

RELATIONSHIP BETWEEN GROWTH OF BACTERIAL FLORA OF MACKEREL AND A RANGE OF TEMPERATURE

Numerous studies have indicated that the range of temperature necessary for the growth of marine forms is lower than is that of most other varieties of bacteria, but it is known that certain nonmarine organisms may grow at temperatures near 0°C.[3]—Prescott and Bates (1931); Prescott, Bates, and Needles (1931); Prescott, Hale, and White (1931); Barber (1908); and Berry (1934).

ZoBell and Feltham (1935), in discussing the occurrence and activity of urea-splitting bacteria in the sea, stated that some of them grew at —4°. ZoBell (1938) reported that most of the organisms from marine bottom sediments studied by him would proliferate at 0° but that their optimum was of the order of 20° in spite of the fact that they were isolated from regions where the temperature of the overlying water was always <5°. The same author (1934) reviewed microbiological activities at low temperatures with particular reference to marine bacteria.

Studies on the bacterial flora of fish have consistently shown that the organisms found in and on fish are able to multiply at low temperatures. Harrison and Sadler (1929), in their study of the discoloration of halibut, noted that the *Pseudomonas fluorescens* which caused the greenish discoloration on the bellies of halibut, grew well at 1.5 to 5°. Gibbons (1933), in studying the slime and intestinal flora of some fishes, used incubation temperatures of 20, 10, and 3° for his mixed cultures before isolation. Growth and pigment formation of *Pseudomonas fluorescens* and *Flavobacterium deciduosum* were obtained by Hess (1934a) at 0, —3, and —6.5°. A most extensive study of the range of temperature required for the growth of marine bacteria is that of Bedford (1933) in which it was demonstrated that nearly all of the cultures isolated from sea water were able to multiply at 0° and that some were able to grow at —7.5° although the optimum was

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[3] All temperatures refer to Centigrade scale.

usually 20 to 25°. Some of these organisms had an optimum temperature of 37° and were able to proliferate at 45°.

EXPERIMENTAL PROCEDURE

Triplicate cultures of 56 strains of bacteria isolated from mackerel were made on ZoBell's agar (1941), and incubated at 20 to 23°, at 7 to 8°, and at 0°. They were examined macroscopically for growth after six days' incubation. All the experiments were confirmed repeatedly.

Eight strains, or 14.4 per cent, failed to show growth macroscopically in six days at 7 to 8°. Of 46 strains incubated at 0° 10.9 per cent, or five, failed to multiply in six days. These 46 did not include any of those which failed to proliferate at 7°. Seventeen, or 39.5 per cent, of a series of 43 cultures incubated at 37° failed to grow in six days, and further incubation at room temperature revealed that they had been killed by this temperature. Sixteen strains chosen from those which grew readily at 0° were tested for their ability to multiply at —4° and all gave positive results.

In view of the fact that six days is a short time for incubation for this low range, it is evident that these organisms are unexpectedly active at these lower temperatures.

EFFECT OF LOW TEMPERATURES OF INCUBATION UPON BIOCHEMICAL ACTIVITIES OF SOME MARINE BACTERIA

These experiments were undertaken to determine whether a decrease in the temperature of incubation of a culture of marine organisms would result in the depression or loss of any of its biochemical activities, i.e., whether one or more sets of enzymatic mechanisms would cease to function. The literature dealing with this question is limited. Hess (1934a) determined the cultural characteristics of *Pseudomonas fluorescens, Flavobacterium deciduosum,* and an unidentified species of *Achromobacter* and reported that most of the reactions of these organisms were retained even at —3° but that at —6.5° many of the activities could no longer be observed although motility and formation of pigment still were retained. Snow and Beard (1939) pointed out that 35 per cent of the strains tested by them for lipolysis at 6.5° were positive in six days but they stated that this result indicated a somewhat greater lipolytic ability than power to produce hydrogen sulfide at that temperature.

EXPERIMENTAL PROCEDURE

Fifteen strains of organisms isolated from mackerel were tested for growth at 20 to 25°, at 7 to 8°, and at —3 to —5° on ZoBell's agar. These included one of *Escherichia,* two of *Micrococcus,* two of *Sarcina,* one of *Streptococcus,* one of *Pseudomonas,* and eight of *Achromobacter.* Triplicate cultures of each organism were made in litmus milk, gelatin, nitrate broth made with sea water, cobalt-nickel agar, and glucose fermentation tubes. One series was incubated at each temperature indicated above. The results were confirmed by repeated series. The cultures at the highest temperature were incubated for two weeks, those at 7° for a month, and those at —4° for two months; results are summarized (Table 1).

TABLE 1

Effect of Temperature on Biochemical Activities of Marine Bacteria

Culture (pct.)	Litmus milk			Gelatin liquefied			Nitrite produced			H_2S produced			Glucose fermented		
	25°C.	7°C.	—4°C.	25°	7°	—4°	25°	7°	—4°	25°	7°	—4°	25°	7°	—4°
3-1	Red.[1]	.....	Sl. Pep.	—[2]	—	—	+	+	0	—	—	0	⊕	⊕	⊕
5-1	Alk. Pep.	Alk. Pep.	Alk. Pep.	+	+	0	—	—	0	—	—	0	+	—	+?
9-1	Red. Pep.	Sl. Alk. Pep.	Alk. Pep.	+	+	0	—	—	0	—	—	—	+	+	0
10-1	Red. Pep.	Alk. Pep.	Alk. Sl. Pep.	+	+	+	—	—	0	?	—	0	—	—	—
12-2	Sl. Pep.	Sl. Pep.	Sl. Pep.	—	—	—	+	—	0	—	0	0	+	+	+
12-4	Alk. Pep. Red.	Red.	Alk.	+	—	—	+	+	+	+	+	0	—	—	—
17-3	Red. Pep.	Red. Pep.	Alk. Sl. Pep.	+	+	+	+	+	+	+	+	—	—	—	?
17-4	Pep.	Red.	Alk. Pep.	+	+	+	+	+	+	—	+	—	—	—	—
17-5	+Red.	Pep.	Pep.	+	+	+	—	—	—	—	—	—	±	—	—
17-6	Alk. Pep.	Red. Pep.	Alk. Pep.	+	+	—	+	+	+	+	+	0	—	—	?
17-7	Red. Pep.	Red. Pep.	Alk. Pep.	+	+	+	+	+	+	+	+	—	—	—	—
17-8	Red. Pep.	Red. Pep.	Alk. Pep.	+	+	+	+	+	+	+	+	—	—	—	—
18-2	Alk. Pep.	Alk. Pep. Sl.	Alk. Pep.	—	0	0	+	+	0	—	—	0	+	0	?
21-1	Pep.	Alk. Pep. Sl.	Alk. Pep.	+	—	0	+	+	0	—	—	0	+	+	—
31-1	—	—	—	—	—	—	—	—	0	—	—	0	±	0	+?

[1] Alk. = alkaline; Pep. = peptized; Red. = reduced; Sl. = slightly. [2] 0 = no growth; — = growth, no reaction; + = reaction indicated by column head; ? = growth questionable; ± = reaction doubtful; ⊕ = acid plus gas.

In a number of instances at the lowest temperature growth failed to appear in the nitrate broth, on cobalt-nickel agar, and in four instances in the gelatin. However, all these strains thrived at —4° on sea-water agar. Strain 12-4 grew in gelatin at all temperatures but failed to hydrolyze it at 7°. All of the strains failed to produce hydrogen sulfide at —4° in the cobalt-nickel agar although certain ones of the series did evolve this by-product at higher temperatures. This result is similar to that obtained by Snow and Beard (1939).

From these results it is to be concluded that growth may not occur in a suboptimal medium at a temperature near the minimum for a given strain and that if growth does occur, not all of the varieties of physiological activities which characterize the strain at the optimum temperature may operate.

A STUDY OF THE GROWTH CYCLE OF A SPECIES OF ACHROMOBACTER AT LOW TEMPERATURES

Investigations regarding the rate of growth of bacteria are almost as old as is bacteriology itself. Buchner, Longard, and Riedlin (1887) reported concerning this subject when they calculated the generation time for the cholera bacillus. Max Müller (1895, 1903) presented a sound basis for further work when he demonstrated the lag phase, the effect of temperature on rate of growth, and the importance of the age of the inoculum.

Lane-Claypon (1909) divided the life cycle of a bacterial culture into four phases when she described the lag, the logarithmic increase, the period of stationary numbers, and a sector of decreasing viable population. She also studied the variation in rate of proliferation with modulation of temperature from 20 to 42° and determined the temperature coefficients of growth for *E. coli, E. typhosa,* and *S. enteritidis.* This work was further elaborated by Penfold (1914) and Ledingham and Penfold (1914) who made an exhaustive study of the lag phase and subjected their experiments to mathematical analysis. Sherman and Albus (1923, 1924) and Sherman and Cameron (1934) studied the effect of adverse influences, such as cold and chemicals, upon *E. coli* in different sectors of the culture cycle and proved that the organisms were most susceptible during the phase of logarithmic increase. Sherman and Naylor (1942) demonstrated that it is possible for the bacteria to pass through the physiological changes described for each phase of the culture cycle without a corresponding alteration in numbers. Hess (1934b) showed that marine organisms are much better adapted to growth at lower temperatures than are others more frequently studied. A summary of the literature dealing with the earlier phases of the cycle of a bacterial culture is contained in the review by Winslow and Walker (1939).

EXPERIMENTAL PROCEDURE

Methods: The organism used in these experiments was an actively proteolytic *Achromobacter* sp. which had been frequently isolated from the stomach, liver, intestine, and muscle of mackerel—Kiser and Beckwith

(1942). The organism was grown in the following medium which was used throughout the experiments:

Bacto-peptone (Difco)	0.5	gm.
Bacto-yeast extract	0.2	gm.
Ferric phosphate, C.P.	0.01	gm.
Bromthymol blue	0.0016	gm.
Filtered sea water	100.0	ml.

This was autoclaved at 15 pounds pressure for 30 minutes and the pH after sterilization was 7.4.

The procedure for the experiments was as follows: A measured amount of the sterile broth was placed aseptically in sterile Erlenmeyer flasks containing glass beads to aid in mixing. The flasks were then fixed in salt-water baths at each incubation temperature and allowed to come to the temperatures of the incubators. These were 25, 7, and —4° plus or minus 1.5°. These flasks were then inoculated with one ml. of a 24-hour broth culture of the organism or with a similar amount of a sea-water dilution of the culture. The amount of the inoculum was determined by plating out the culture in duplicate on an agar medium of the composition of the broth with addition of 1.5 per cent Bacto-agar (Difco). The plates were incubated six days at room temperature. Counts were made of each one of the cultures at intervals and the necessary dilutions were compounded in a sterile sea water.

The experiment indicated (Table 2 and Figs. 1 to 4) is representative and differed from others only in the amount of inoculum. In the first experiment the initial count was 5.38×10^2, in the second it was 2.86×10^4, and in the fourth it was 2.97×10^6. Experiments 2 and 3 were similar except that the culture used for starting the flasks in Experiment 3 was carried for a month prior to its use at 7° rather than at room temperature. A 96-hour culture was used for the inoculum in Experiment 3 and the initial number of bacteria was 3.20×10^4. Inasmuch as similar results appeared in all of these tests the figures which pertain to only one of them are included (Table 2).

Results: The general shape of the curves formed by plotting the logarithms of the number of bacteria per milliliter against time in hours was similar in all four cases as shown (Figs. 1 to 4) except for the dip in the curve for 25° in Fig. 4. For this dip no explanation is offered.

The maximum numbers of viable bacteria held at 25° were less than those at 7° and the flasks sewn with the smaller inocula yielded results substantially the same. However, these were larger than those obtained from the flask started with the largest inoculum. The maximum in the preparation held at —4° was approximately the same throughout its series, although the maximum viable population in the case of the flask with the smallest inoculum was not reached during the period of the particular experiment detailed (Table 2). The total numbers of bacteria as judged by turbidity were considerably greater at the two higher temperatures regardless of initial numbers than in the flasks incubated at —4°. The maximum number at 7° was slightly greater than that at 25° in each instance.

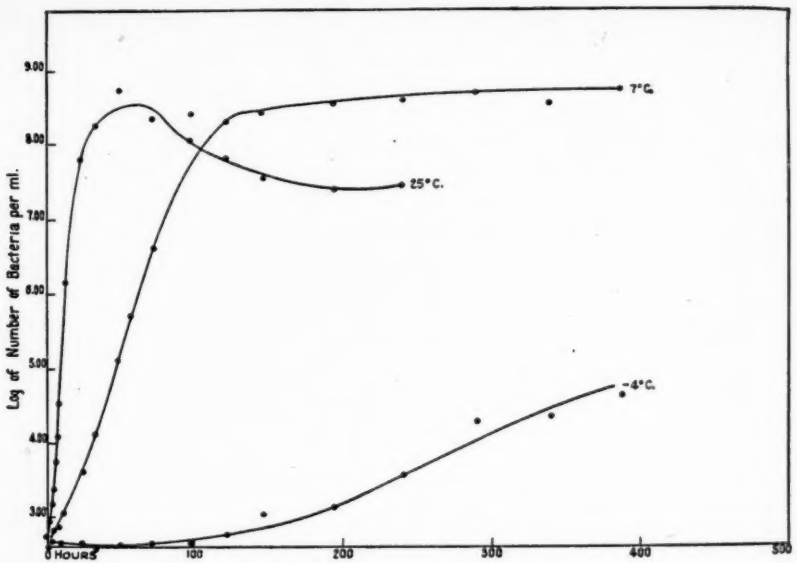

FIG. 1. Growth curves—5.38×10^2 inoculum.

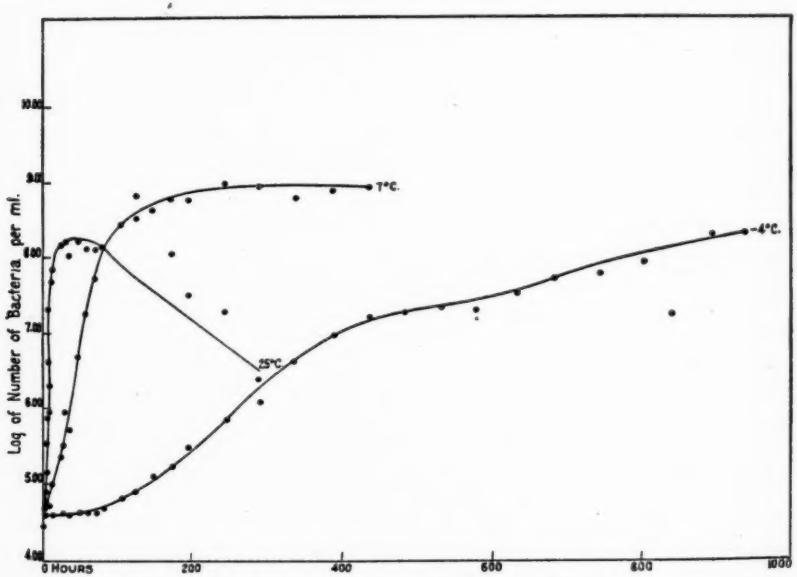

FIG. 2. Growth curves—2.86×10^4 inoculum.

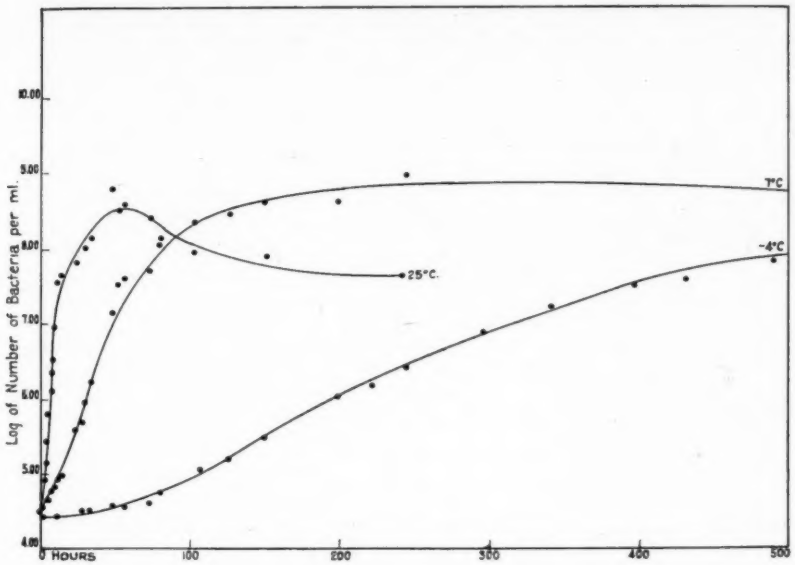

FIG. 3. Growth curves—3.20×10^4 inoculum.

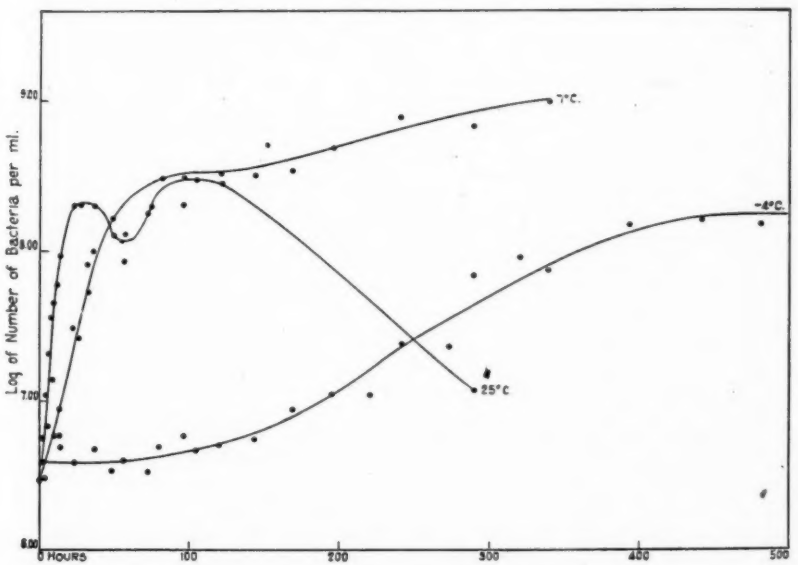

FIG. 4. Growth curves—2.97×10^6 inoculum.

The lag period at 25° approximated three hours (Table 3). At 7° the lag was 24 to 28 hours except in the fourth experiment where it was 12 hours. This experiment included the largest inoculum and its result is in agreement with those of Rahn (1906) and of Penfold (1914), who showed that the lag was inversely proportional to the size of the inoculum. At —4° the lag with a small inoculum was 193 hours but if this inoculum was 100-fold larger, it was 125-127 hours. With the largest amount of starter it was 144 hours. At this low temperature much difficulty was experienced with icing. Likewise it appeared at the beginning of the fourth experiment and during the period of 450 to 700 hours in Experiment 2. The cultures have a freezing point of 3° but the medium ordinarily supercooled and no ice formed. When ice formed, growth was slowed.

TABLE 2

Growth of an Achromobacter sp. at 25, 7, and —4°C. With Inoculum of 5.38×10^{6}

Time	25°C.		7°C.		—4°C.	
	Bacteria per ml.	Log	Bacteria per ml.	Log	Bacteria per ml.	Log
hr.						
0	5.38×10^{2}	2.73	5.38×10^{2}	2.73	5.38×10^{2}	2.73
1	3.75×10^{2}	2.57	.....	.....	.....	.....
2	5.60×10^{2}	2.75	4.95×10^{2}	2.69	.....	.....
3	9.40×10^{2}	2.97	.....	.....	.....	.....
4	1.48×10^{3}	3.17	.....	.....	4.60×10^{2}	2.66
5	2.38×10^{3}	3.38	.....	.....	.....	.....
6	5.70×10^{3}	3.76	6.75×10^{2}	2.83	.....	.....
7	1.24×10^{4}	4.09	.....	.....	.....	.....
8	3.44×10^{4}	4.54	.....	.....	.....	.....
9	.....	.....	7.40×10^{2}	2.87	.....	.....
10	.....	.....	.....	.....	4.40×10^{2}	2.64
12	.....	.....	1.14×10^{3}	3.06	.....	.....
14	1.42×10^{6}	6.15	.....	.....	.....	.....
24	6.40×10^{7}	7.81	4.10×10^{3}	3.61	4.75×10^{2}	2.68
33	1.85×10^{8}	8.27	1.28×10^{4}	4.11	3.85×10^{2}	2.59
49	5.35×10^{8}	8.73	1.23×10^{5}	5.09	4.10×10^{2}	2.61
57	.....	.....	5.09×10^{5}	5.71	.....	.....
72	2.19×10^{9}	8.34	4.06×10^{6}	6.61	4.50×10^{2}	2.66
98	2.50×10^{9}	8.40	1.12×10^{8}	8.05	4.50×10^{2}	2.65
121	6.45×10^{7}	7.81	2.08×10^{8}	8.32	5.70×10^{2}	2.76
146	3.45×10^{7}	7.54	2.60×10^{8}	8.42	1.10×10^{3}	3.04
193	2.40×10^{7}	7.38	3.45×10^{8}	8.54	1.45×10^{3}	3.16
240	2.75×10^{7}	7.44	3.90×10^{8}	8.59	3.90×10^{3}	3.59
289	.....	.....	4.65×10^{8}	8.67	2.00×10^{4}	4.30
339	.....	.....	3.45×10^{8}	8.54	2.24×10^{4}	4.35
386	.....	.....	5.50×10^{8}	8.74	4.35×10^{4}	4.64

The minimum generation times were computed for each experiment by use of the formula of Buchner, Longard, and Riedlin (1887):

$$g = \frac{\log 2\,t}{\log b - \log B}$$

Here B is the number of bacteria at the beginning and b is the number at the end of the time t; g is the time of generation.

At the higher temperatures the minimum generation time (M.G.T.) is considerably less with the two smaller inocula than it is with the largest but at —4° the range of M.G.T. is greater and there is no apparent correlation between the size of the inoculum and M.G.T.

At 25° the length of time during which the M.G.T. is maintained is greatest with the smallest inoculum and is shortest with the largest, as would be expected if the extent of growth were controlled by the numbers of bacteria or by the concentration of metabolic products. At 7° the period during which the M.G.T. was maintained was shorter in the third experiment than it was in Experiment 2 but the M.G.T. was considerably less. This suggests that some adaptation to the lower temperature had occurred during the period when the stock culture was maintained at 7°.

The activity coefficient (K) in terms of production of bacteria per unit of time was computed from the formula:

$$K = \frac{2.3}{t} (\log b - \log B)$$

where b is the final and B the initial number of bacteria per milliliter after time t. K is also equal to $\frac{0.692}{g}$ and thus could be computed directly from g if desired. Having obtained a value for K it is possible to compute the temperature coefficient for the growth process. This was done by means of the formula of Kanitz (cited by Hess, 1934b):

$$Q_{10°} = 10 \frac{(10(\log K_2 - \log K_1)}{T_2 - T_1}.$$

Between 7 and 25° there appears a two to threefold increase in growth for each 10° rise in temperature (Table 4), and this is comparable to the

TABLE 3

Generation Times and Activity Coefficient of Achromobacter sp.

Exp. No.	Hours	Elapsed time in hours	g	k
		25°C.		
I	3-14	11	1.05	0.663
II	4-10	6	0.830	0.835
III	3-10	7	1.05	0.663
IV	4-6	2	2.15	0.322
		7°C.		
I	24-72	48	4.83	0.144
II	28-60	32	5.47	0.127
III	25-34	9	4.23	0.164
IV	12-33	21	6.57	0.105
		—4°C.		
I	193-290	97	25.6	0.0270
II	125-244	110	38.1	0.0181
III	127-244	117	28.4	0.0244
IV	144-290	146	40.0	0.0174

effect of temperature upon many chemical reactions at this range. However, between —4 and 7° there is a five or sixfold increase in growth for each rise of 10° in temperature. This is a much smaller value for $Q_{10°}$ than those previously reported by Hess (1934b). It does not seem likely that the amount of inoculum is significant here.

TABLE 4

Temperature Coefficient for a Ten-Degree Rise in Temperature

Exp. No.	25 to 7°C.	7 to —4°C.
I	2.17	5.65
II	2.84	5.82
III	1.86	5.12
IV	2.33	4.58

SUMMARY

The organisms isolated from the mackerel were well adapted to growth at low temperatures. Eighty per cent of the cultures produced macroscopically visible growth in six days at 0°.

These organisms did not always prolificate in all media used at nearly minimal temperatures and when they did grow they did not always carry on the same physiological activities which were characteristic at temperatures more nearly optimal.

Growth curves for an *Achromobacter* sp. were obtained at three temperatures, 25, 7, and —4°, by use of inocula which varied between 5.38×10^2 and 2.97×10^6 bacteria per milliliter.

When the stock culture was incubated at 7° for one month there was an adaptation to growth at that temperature.

The average minimum generation times for the three smaller inocula were: 25°, 0.98 hour; 7°, 4.84 hours; —4°, 30.7 hours. These generation times indicate a much more rapid rate of growth at the two lower temperatures than has been reported for most other bacteria.

The temperature coefficient for an increase in temperature of 10° is about the same as that for many chemical reactions, i.e., 1.86 to 2.84 at the higher temperatures tested. In the lower range of temperature, between 7 and —4°, the temperature coefficient is somewhat greater, i.e., from 4.58 to 5.82. This is much less than the figures usually reported for the biological phenomena apparent in that range of temperature.

ACKNOWLEDGMENT

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ASCORBIC ACID CONTENT, COLOR, AND PALATABILITY OF FRESH AND PROCESSED SWISS CHARD AND BEET GREENS[1,2]

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This paper deals with certain phases of a study which is part of a national co-operative project on the "Conservation of the Nutritive Value of Foods." The purpose of the investigation was threefold: (1) to learn the relative nutritive values of some horticultural varieties of *Beta vulgaris* which seemed to offer possibilities of increased utilization as human food; (2) to determine their relative palatabilities; and (3) to investigate the effect of cooking, marketing, and drying on the ascorbic acid content, color, and palatability of the selected greens.

Much has been published on the ascorbic acid content of vegetables and the effect of processing on it but little has been done with leaves of the *Beta vulgaris* family. Fenton, Tressler, Camp, and King (1937) found the total ascorbic acid content of fresh raw Fordhook chard to be 3.6 mg. per gram (dry weight) by the dye-titration method. Sheets, Leonard, and Gieger (1941) and Fenton *et al.* (1937) collected data which showed that the leaf portion of raw fresh Swiss chard was a good source of vitamin C, while the stem was a poor source. Sheets *et al.* (1941) found that the leaf blade contained 86.6 per cent of the ascorbic acid of the entire leaf.

The ascorbic acid content of cooked Swiss chard was found by Fenton *et al.* (1937) to be 0.14 to 0.18 mg. per gram with about one-half of the original ascorbic acid passing into the cooking water. Fenton (1940), in reviewing the published data, stated that the amount in cooking water varied from 20 to 66 per cent. McIntosh, Tressler, and Fenton (1940) found that quick-frozen vegetables lost increasing amounts of vitamin C to the cooking water as the volume of the water was increased. Fenton *et al.* (1938) working with carrots had similar results. Most of the actual destruction of ascorbic acid occurred during first two minutes of cooking.

Nagel and Harris (1943) found that high losses of ascorbic acid occurred when vegetables were cooked and served under restaurant conditions. Their data indicated that about 20 per cent of the original ascorbic acid content was destroyed by cooking, 25 per cent more was lost in the discarded cooking water, and an additional 25 per cent was lost during approximately three hours on the steam table. In spinach, the total loss was 93 per cent.

Tressler (1942) stated that most leafy vegetables lost their vitamin C content during dehydration. Moyer (1943) reported that beets lost 30 per

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[2] Paper presented before Division of Biological Chemistry of the American Chemical Society, Detroit, Mich., April 13, 1943.

cent, rutabagas 85 per cent, and potatoes almost all, on dehydration. Storage above —40°C.(—40°F.) accelerated the loss of ascorbic acid.

EXPERIMENTAL PROCEDURE

Leaves of different varieties of the beet family (*Beta vulgaris*) were selected for study. These included four horticultural selections: the common table beet (variety, Detroit Dark Red); the sugar beet (variety, U. S. 200 × 215); and two varieties of Swiss chard successfully grown in Michigan—Fordhook, a dark green, curly-leaf variety, and Rhubarb, a red-stemmed variety. The greater part of the work was carried out on Swiss chard. The leaves of the Detroit Dark Red beet were obtained from a local truck gardener and the sugar beets were grown on college plots. Under the direction of the Horticultural Department, the two varieties of chard were planted side by side and received the same cultural practices. This controlled method of production should validate comparisons of the findings on these two kinds of greens.

Early on the day that the experiments were conducted, the greens were harvested according to a standard procedure. Leaves representing the partially mature growth of the plant were used. Thirty minutes after picking, the leaves were in the laboratory for analysis. Harvesting of the beet leaves was done in July and the Swiss chard in August and September, 1942. The August and September harvesting are referred to as Periods 1 and 2, respectively. Fresh and processed leaves were analyzed with midribs but without petioles, since this seemed to be a generally accepted method of preparation for home consumption in this part of the country. An aliquot of freshly picked leaves was removed as a control from each lot to be processed. All samples of greens were analyzed in triplicate. The fresh leaves were quickly rinsed in distilled water, dried with cheesecloth, clipped with shears into approximately one-cm. squares, dropped into a paper bag, and shaken to mix. Samples were weighed at once for the analyses.

The greens were prepared for cooking by removing undesirable leaves, cutting off petioles, and washing three times in tap water at 20°C.(68°F.). The *home-cooking* procedures were as follows: Greens equivalent to four servings were cooked (1) uncovered, in twice their weight of rapidly boiling tap water, and (2) covered, without the addition of water except that clinging to the leaves after one minute of draining. Three-quart, enamel saucepans were used for cooking. Since the purpose of this study was to determine the nutritive value of the leaves as eaten, no analysis of the cooking water was made. *Institution preparation* was carried out by cooking greens in eight-lb. lots (approximately 30 servings) in a steam-jacketed kettle in double their weight of rapidly boiling water for seven minutes. Cooked greens were held on the steam table for 10 and 60 minutes, respectively. The *home-drying* method consisted of steam-blanching the leaves for five minutes and then drying with gas-heated air in home-sized driers made of plywood and galvanized sheet iron, according to Caldwell (1933) and Case (1940). The leaves were dried at a temperature under 70°C.(158°F.) for about five hours or until they contained from seven to 10 per cent moisture, and then were stored in sealed cellophane

FIGURE 1

Grading Chart for Vegetables

Code No._____ Date_____

Factor	7	6	5	4	3	2	1	Remarks
Odor	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable	
Color	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable	
General appearance	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable	
Consistency	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable	
Flavor	Very desirable	Desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Undesirable	Very undesirable	
General conclusion	Excellent	Very good	Good	Medium	Fair	Poor	Very poor	

Check the appropriate adjectives in each column.

Odor	Color	Consistency	Flavor
1. Grassy	1. Brown green	1. Chewy	1. Bitter
2. Hay-like	2. Faded green	2. Crisp	2. Grassy
3. Moldy	3. Grey green	3. Firm	3. Hay-like
4. Musty	4. Natural green	4. Hard	4. Lacking
5. Natural	5. Purple	5. Limp	5. Musty
6. Pungent	6. Yellow green	6. Leathery	6. Natural
7. Sour		7. Mushy	7. Pungent
8. Stale		8. Soft	8. Sour
9. Strong		9. Tender	9. Strong
10. Sweet		10. Tough	10. Stale
			11. Sweet
			12. Weak
			13. Weedy

Name_____

bags in a cupboard at approximately 18° C. (64.4° F.). The *marketing* procedure consisted of taking a portion of the freshly harvested leaves, rinsing in a tub of water, covering with wet burlap in bushel baskets, and allowing to stand at room temperature for 24 hours. Ascorbic acid and moisture determinations were made and compared with the values obtained the previous day from the freshly picked sample. After cooking, processed samples were scored for palatability by four judges using a seven-point rating scale (Fig. 1) on the factors of color, odor, general appearance, consistency, flavor, and general conclusions.

For analytical purposes the samples of the cooked greens were taken by means of glass boring tubes, one-half inch in diameter, pressed through a well-mixed and compact layer of greens. Moisture samples were run parallel with the vitamin determinations, the leaves being dried at 95 to 105° C. (203 to 221° F.) for 24 hours. Reduced ascorbic acid was determined by Morell's (1941) modification of the Bessey (1938) procedure using a Coleman universal spectrophotometer, Model 11.

The color of the cooked product was determined by the Petering, Wolman, and Hibbard (1940) procedure for chlorophyll. It is referred to as alcohol-extracted pigment rather than chlorophyll since the cooked material would contain degradation products of chlorophyll as well as the pure pigment.

When the number of samples permitted, mean vitamin values were analyzed by variance methods for statistically significant differences. Correlation values between the alcohol-extracted pigment and (a) the ascorbic acid content, (b) the color scores, and (c) the general palatability scores were determined.

TABLE 1

Ascorbic Acid Content of Freshly Picked Leaves

Variety of leaves	Number of samples	Moisture	Ascorbic acid	
			Fresh leaves	Calculated to dry basis
Swiss chard		*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
Fordhook	24	91±0.2	35±1.6	376±19.7
Rhubarb	24	89±0.3	39±1.8	369±13.2
Beet leaves				
U. S. 200×215	6	88±0.3	62±2.2	504±23.9
Detroit Dark Red	3	90±0.3	61±0.8	585± 7.9

RESULTS AND DISCUSSION

The fresh leaves of *Beta vulgaris* contained about 90 per cent moisture and, in ascorbic acid content, ranged from 35 to 62 mg. per 100 gm., as shown (Table 1). (In this table the results from the two harvesting periods for Swiss chard were averaged since there was no significant difference between them.) In the raw state both varieties of beet leaves were better sources of ascorbic acid than were the varieties of Swiss chard. The beet tops contained 61 to 62 mg. per 100 gm. (fresh weight), while the Swiss chard leaves ranged from 35 to 39 mg. Compared with published data of Booher, Hartzler, and Hewston (1942), the beet-top values were high. These greens were picked in July. The average ascorbic acid content for

TABLE 2

Effect of Home-Cooking Methods on Ascorbic Acid Content of Greens[1]

Variety and period	Ascorbic acid in fresh raw sample (dry wt.)	Method 1[2]				Method 2[3]			
		Moisture	Ascorbic acid values			Moisture	Ascorbic acid values		
			As served	Dry	Loss		As served	Dry	Loss
	mg./100 gm.	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Swiss chard									
Fordhook									
Period 1	406±10.1	92±0.6	4.1±0.2	48± 2.5	88	90±0.3	2.8±0.4	27±4.2	93
Period 2	416±30.3	91±0.3	7.4±0.6	84± 8.5	80	89±0.2	2.3±0.3	20±2.3	95
Rhubarb									
Period 1	383±25.0	90±0.1	6.4±0.3	67± 2.8	83	88±0.5	2.1±0.1	19±0.9	95
Period 2	325±33.9	91±0.3	7.4±0.6	85±10.0	74	89±0.4	2.1±0.7	20±6.8	94
Beet leaves									
U. S. 200×215	504±23.9	90±0.3	17.6±1.0	174± 8.3	65	86±0.3	11.6±1.1	84±7.9	83
Detroit Dark Red	447±61.3	93±0.2	9.0±1.6	129±22.9	71	89±0.2	8.1±2.2	71±18.9	84

[1] Each figure represents an average of six determinations. [2] Cooked in a moderate quantity of rapidly boiling water. [3] Cooked in water clinging to the leaves after the last rinsing.

TABLE 3

Effect of Institution-Cooking Methods on Ascorbic Acid Content of Greens[1]

Variety and period	Ascorbic acid in fresh raw sample (dry wt.)	Method 1[2]				Method 2[3]			
		Moisture	Ascorbic acid values			Moisture	Ascorbic acid values		
			As served	Dry	Loss		As served	Dry	Loss
	mg./100 gm.	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Swiss chard									
Fordhook									
Period 1	422±12.8	90±0.2	6.3±0.4	64±3.9	85	89±0.6	1.8±0.3	16±3.0	96
Period 2	262±46.4	92±0.2	4.6±0.2	54±0.2	79	90±0.2	0.7±0.1	7±1.4	97
Rhubarb									
Period 1	407±20.0	90±0.3	8.8±0.3	86±3.3	79	88±0.4	2.3±0.3	19±2.9	95
Period 2	361±14.2	91±0.2	4.9±0.1	55±1.5	85	91±0.2	1.3±0.3	14±2.2	96
Beet leaves[4]									
U. S. 200×215	423±19.2	87±0.3	8.4±0.5	66±3.3	84	88±0.2	4.4±0.2	36±1.2	91
Detroit Dark Red	193±19.0	91±0.1	4.3±0.8	45±8.2	77	90±0.4	2.5±0.1	25±3.1	87

[1] Each figure represents an average of six determinations. [2] Held on steam table 10 minutes. [3] Held on steam table 60 minutes. [4] Beet leaves, U. S. 200×215, were cooked for 16 minutes and Detroit Dark Red for 12 minutes in a pressure steamer. The steam-table treatment was the same as that for the Swiss chard.

Fordhook chard, 35 mg. per 100 gm. of fresh leaves, was in agreement with Booher's figures, which ranged from 32 to 38 mg. per 100 gm. of fresh greens. Rhubarb chard averaged 39 mg. per 100 gm. of fresh leaves. No values were found in the literature for the Rhubarb variety. There were no statistically significant differences, however, in the ascorbic acid values of the fresh leaves of the two varieties of Swiss chard as observed in this study.

Marked decreases were noticeable in ascorbic acid when the greens were cooked by either of the home-cooking methods (Table 2). The data for each set of cooked samples were compared with those from a corresponding set of fresh aliquots. With Swiss chards there were losses ranging from 74 to 88 per cent when the greens were cooked in a moderate quantity of rapidly boiling water (Method 1). Time for cooking to a tender stage was seven to eight minutes by this method. Even greater losses of ascorbic acid were observed in Swiss chards cooked in the water clinging to the leaves after the last rinsing (Method 2), those losses ranging from 93 to 95 per cent. Time for cooking to a tender stage by Method 2 was 21 to 22 minutes. The beet greens showed losses of from 65 to 71 per cent by Method 1, and from 83 to 84 per cent by Method 2. While the preservation of ascorbic acid in cooked greens was significantly greater by Method 1 than by Method 2, in either case it was less than that observed by Fenton *et al.* (1937). No analyses were done on cooking water in this study, since the greens were assayed "as eaten." The preservation of slightly more ascorbic acid in leaves cooked in a moderate quantity of rapidly boiling water than in leaves cooked in no added water, is probably due to the shorter cooking time or to the shorter time required to bring the leaves to the boiling point.

Cooking greens under conditions prevailing in the institution kitchen, at least as used in this study, and "holding" them for 10 minutes on a steam table resulted in losses of from 77 to 85 per cent of their ascorbic acid (Table 3). Holding for 60 minutes on the steam table caused significantly greater losses, ranging from 87 to 97 per cent.

The effects of home and institution cooking on palatability and color of greens are presented (Table 4). Judges' scores showed that cooking greens in a moderate quantity of rapidly boiling water was superior to that of cooking with no added water. Fordhook and Rhubarb chards received higher scores for palatability than beet greens. The palatability scores were somewhat lower for institution cooked greens than for greens cooked by Method 1 of home cooking. Holding these greens for 10 or for 60 minutes on a steam table made no significant change in the final scores on palatability. In this connection, it should be pointed out that the factor of time was not as well managed as desired, since a period of from 20 to 30 minutes was required for transfer of the cooked greens from the institution kitchen in one of the dormitories to the laboratories. This period may have represented a critical period so far as the palatability of the greens was concerned.

There was a highly significant correlation between the alcohol-extracted pigment and either palatability or color as scored by the judges. It is possible that the determination of alcohol-extracted pigment as car-

Detroit Dark Red	193±19.0	91±0.1	4.3±0.8	45±8.2	77	90±0.4	2.5±0.1	25±3.1	87

[1] Each figure represents an average of six determinations. [2] Held on steam table 10 minutes. [3] Held on steam table 60 minutes. [4] Beet leaves, U. S. 200×215, were cooked for 15 minutes and Detroit Dark Red for 12 minutes in a pressure steamer. The steam-table treatment was the same as that for the Swiss chard.

TABLE 4

Effect of Home and Institution Cooking on Palatability and Color of Greens[1]

Variety and period	Home cooking						*Institution cooking*					
	Method 1[2]			Method 2[3]			Method 1[4]			Method 2[5]		
	Palatability (general conclusions)	Color as scored by judges	Alcohol-extracted pigment	Palatability (general conclusions)	Color as scored by judges	Alcohol-extracted pigment	Palatability (general conclusions)	Color as scored by judges	Alcohol-extracted pigment	Palatability (general conclusions)	Color as scored by judges	Alcohol-extracted pigment
Swiss chard												
Fordhook												
Period 1	5.2	6.2	1288	2.8	2.8	983	4.8	5.7	1573	4.4	5.3	1294
Period 2	5.8	6.4	1477	2.1	2.8	970	5.3	5.8	1287	4.1	5.6	1031
Rhubarb												
Period 1	5.0	6.4	1515	3.4	5.2	1026	4.6	6.3	1700	4.3	5.7	1379
Period 2	5.2	6.2	1342	1.9	3.6	902	4.5	6.4	1452	4.2	5.2	1499
Beet leaves[6]												
U. S. 200×215	4.2	6.1	1264	1.8	3.1	871	2.7	3.7	737	2.9	3.8	786
Detroit Dark Red	4.8	6.1	1584	3.2	4.4	955	2.9	4.3	1030	2.9	4.6	1025

[1] Each figure represents an average of four scores by each of four judges for Swiss chard and an average of two scores by each of four judges for beet leaves. The alcohol-extracted pigment represents an average of six determinations. [2] Cooked in a moderate quantity of rapidly boiling water. [3] Cooked in water clinging to the leaves after the last rinsing. [4] Held on steam table 10 minutes. [5] Held on steam table 60 minutes. [6] Institution methods—beet leaves, U. S. 200×215, were cooked for 16 minutes and Detroit Dark Red for 12 minutes in a pressure steamer. The steam-table treatment was the same as for the Swiss chard.

ried out in this study might be developed as an objective method of measuring certain factors contributing to the table quality of green vegetables.

The home-drying method used in this experiment resulted in practically complete disappearance of reduced ascorbic acid from the greens (Table 5). In all varieties except Fordhook the amount remaining was too small to be determined by the method used. Other workers, Moyer (1943) and Tressler (1942), have reported somewhat similar findings. The dried greens, when reconstituted and cooked, had a good color, were not unattractive, but were extremely unpalatable and of very doubtful practical value in the diet either from the point of view of appetite appeal or nutritive value.

TABLE 5

Effect of a Home-Drying Method on Ascorbic Acid Content of Greens

Variety and period	Number of samples	Moisture	Ascorbic acid		
			Fresh raw sample (dry basis)	Newly dried (dry basis)	Loss
Swiss chard		*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Fordhook					
Period 1	6	7.3±0.4	408±23.0	16±1.5	96
Period 2	3	7.9±0.1	460±40.4	14±0.6	97
Rhubarb					
Period 1	6	10.0±0.2	436±13.7	0	100
Beet leaves					
U. S. 200×215	3	10.3±0.1	460±20.8	0	100
Detroit Dark Red	3	6.0±0.001	585± 7.9	0	100

Holding the greens for 24 hours in a moist condition at room temperature did not cause any significant loss of reduced ascorbic acid. As shown (Table 6), with Fordhook chard there were losses but with the Rhubarb chard there was an apparent gain in ascorbic acid. In every case the leaves were in excellent condition. Nothing has been found in the literature concerning the procedure of holding washed, moist greens in

TABLE 6

Effect on Ascorbic Acid Content of Holding Greens for 24 Hours Under Local Market Conditions[1]

Variety and period	Moisture of "held" leaves	Ascorbic acid		
		Freshly picked (dry basis)	Held 24 hours (dry basis)	Change from fresh values
Swiss chard	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Fordhook				
Period 1	91±0.1	396±14.1	366± 8.4	— 8
Period 2	92±0.1	416±30.4	255±16.0	—39
Rhubarb				
Period 1	90±0.3	347± 9.2	375±20.1	+ 8
Period 2	91±0.5	325±33.9	354±47.1	+ 9

[1] Each figure represents an average of six determinations.

baskets under wet burlap; but, since this method was practiced in the local farmers' market, it was considered worthy of investigation.

SUMMARY

A study was made of the ascorbic acid content and palatability of leafy vegetables as affected by (1) variety, (2) cooking by two widely used home-cooking methods, (3) by holding the greens cooked by an accepted institutional method for (a) 10 minutes and (b) 60 minutes on a steam table, (4) home drying, and (5) marketing as on the local farmers' market. All varieties of leaves were from the beet family (*Beta vulgaris*)—the common table beet (variety, Detroit Dark Red), the sugar beet (variety, U. S. 200×215), and two varieties of Swiss chard (Fordhook and Rhubarb). The beet leaves were analyzed in July and the Swiss chard at two periods, in August and September.

The average ascorbic acid content for Fordhook was 35 mg. per 100 gm. of fresh leaves; Rhubarb, 39 mg. per 100 gm.; beet leaves (U. S. 200 ×215), 62 mg. per 100 gm.; and beet leaves (Detroit Dark Red), 61 mg. per 100 gm.

There was no statistically significant variation in the level of ascorbic acid between the August and September harvesting of Swiss chard.

Cooking in family-size quantities was more desirable when done in a moderate quantity of rapidly boiling water than when done with no water except that clinging to the leaves after the last rinsing. From a statistical analysis, the former method was superior for the preservation of color, for the retention of reduced ascorbic acid, and for palatability.

The institution cooking under the conditions of the experiment produced greens low in ascorbic acid. Holding the greens cooked by the institution method for 10 or 60 minutes seemed to make little difference in the final scores on palatability, but the reduced ascorbic acid was significantly lowered after the 60-minute holding period

Both varieties of Swiss chard ranked higher on palatability scores than either the red-beet or sugar-beet leaves.

Drying in a small home drier resulted in greens with very low, or no, reduced ascorbic acid content. The dried leaves were unpalatable when reconstituted and cooked.

The alcohol-extracted pigment gave promise of being a good objective test for desirability and palatability of the cooked product.

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SALT MIGRATION IN CHEDDAR CHEESE CURD AND ITS EFFECT ON MOISTURE CONTENT, pH, AND BACTERIAL CONTENT[1]

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In salting Cheddar cheese, granular salt is added to the surfaces of the freshly milled pieces of curd. The salt dissolves in the moisture on or near the surfaces of the pieces of curd and, while a portion of the salt is removed in the whey that drains away, a large portion of it diffuses from the surfaces to the interiors of the pieces. Since salt has a retarding effect on bacterial action, the time required for appreciable amounts of salt to migrate from the surfaces to the centers of the pieces of curd may be biologically significant. Also, differences in the concentrations of salt at various points in the pieces of curd may affect the moisture distribution.

In the studies herein reported moisture and salt determinations were made on micro-samples taken from the surfaces and centers of pieces of curd at intervals during the first 72 hours after hooping, which commonly was as long as the pieces retained their identity in the cheese. Variations in bacterial action were studied by determining the pH values and bacterial contents of samples from the surfaces and centers of the pieces.

GENERAL PROCEDURE

The cheese used in the studies were made in small experimental vats from 380-pound lots of pasteurized milk, using the procedures generally employed in the cheese laboratory for making Cheddar cheese from pasteurized milk, Wilson (1943). Approximately 2.5 per cent granular salt was added to the milled curd in three portions at intervals of approximately 15 minutes. About one hour after the first addition of salt, the curd was rinsed with a small quantity of water at approximately 38°C. (100.4°F.) and then placed in longhorn type hoops. The cheese were pressed for 48 hours after which they were removed and held at about 10°C. (50°F.).

Whole pieces of curd were obtained from a cheese by picking away the surface and selecting pieces from beneath the surface. A cheese was sampled at 0, 5, 15, 24, and 48 hours after being placed in the press and also 24 hours after removal from the press. From each piece of curd portions were taken at the surface and from the interior, and the moisture, salt, and pH values were determined. Moisture and salt determinations were made on the same micro-sample; pH determinations were made on samples of considerable size from the piece of curd already used for moisture and salt analyses. The final values represent the averages of the values obtained on surface and center portions from two pieces of curd. Bacterial contents were determined on one-gram portions taken at the surface and center of a separate piece of curd.

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MOISTURE AND SALT DETERMINATIONS

The sampling of a piece of curd was done as quickly as possible to minimize evaporation. Under a microscope having X6 magnification, a sample of approximately one to five mg. was obtained with a sharp razor blade. The sample was placed on a weighed micro-spoon [2] and then the micro-spoon was immediately placed in a glass-stoppered micro-weighing bottle (seven mm. in diameter and 11 mm. in height). All weighings were made in the micro-weighing bottle and the weights determined to within 0.002 mg. on an Ainsworth micro-balance. No loss in moisture was noted during the weighing.

For the moisture determinations, the samples were dried at 100°C. (212°F.) for 24 hours and then placed in a desiccator for 20 minutes before reweighing. The loss in weight was considered to represent the moisture in the sample.

The micro-method for salt was essentially the volumetric micro-procedure described by Ogg, Johns, Hoecker, and Hammer (1942) for determining salt in butter, although a few modifications were employed. The general method consisted of weighing the sample; emulsifying the curd with sodium hydroxide; transferring the ash and salt to a spot plate with water, a buffer solution, and alcohol; and titrating with a 0.01N silver nitrate solution, dichlorofluorescein being used as the indicator.

Before ashing a sample, one drop of 0.1N sodium hydroxide solution was added to it in the micro-spoon. The sample was then heated in a micro-oven to approximately 125°C.(257°F.) until it was apparently dry. Heating the curd with sodium hydroxide tended to emulsify the curd, thus causing it to ash more easily. The sample was ashed by hanging the micro-spoon, containing the sample, on a Nichrome wire suspended in a 15-ml. porcelain crucible. The crucible was heated slowly with a micro-burner until the sample was thoroughly charred, after which the crucible was covered and the heating continued until only the ash and salt remained (approximately 10 to 15 minutes).

The ash and salt were transferred to a white porcelain spot plate by placing the spoon in several drops of water. After a few minutes the salt solution was washed from the spoon with three or four drops of water followed by two drops of an ammonium acetate buffer solution [one part by volume of 1M acetic acid solution and two parts by volume of 1M ammonium acetate solution, according to Benedetti-Pichles and Siggia (1942)] and finally with three or four drops of 95-per cent ethyl alcohol. When the salt concentration in the solution from the ash was relatively low, a measured amount of 0.01N sodium chloride solution was added from a micro-buret. The titration with silver chloride was then carried out as described by Ogg *et al.* (1942).

Macro-procedures also were used in determining the moisture and salt contents of some of the cheese. A representative sample of a cheese was put through a food chopper several times and thoroughly mixed. For the moisture determination a three to four gram sample was weighed in a

[2] The micro-spoons were made by fusing pieces of platinum foil about six mm. in diameter to 24-gauge platinum wires about 10 mm. in length. The centers of the spoons were made slightly concave and the wires were bent so that the spoons would hang level.

tared aluminum dish, containing a small amount of sand, and dried in an oven at 100°C.(212°F.) for 48 hours; the percentage of moisture was calculated from the loss in weight. Salt was determined by the A.O.A.C. official method (1940).

pH DETERMINATIONS

A sample was obtained from the surface of a piece of curd by cutting a very thin slice from each of the faces and from the center by splitting the piece of curd and cutting a relatively small portion from as near the center as possible. Each sample was thoroughly macerated in a small glass mortar after which a small quantity of quinhydrone was mixed with it. The mixture was transferred to a small glass sampling tube by tapping one end of the tube in the mixture. The pH was determined with a Leeds and Northrup potentiometer, a calomel half-cell, and a modified quinhydrone electrode similar to the one described by Watson (1929).

BACTERIAL COUNTS

Bacterial counts were made by the plate method, using tryptone-glucose-extract-milk agar, American Public Health Association (1941), and an incubation of five days at 21°C.(69.8°F.). A one-gram sample of the curd was thoroughly macerated in a sterile mortar and then ground with nine ml. of sterile two-per cent sodium citrate solution. The mixture was transferred to a warm, sterile, 90-ml. water blank and the desired dilutions were made.

EXPERIMENTAL

ACCURACY OF MICRO-PROCEDURES FOR MOISTURE AND SALT DETERMINATIONS ON CHEESE

The accuracy of the micro-procedures for moisture and salt determinations on cheese could not be evaluated by analyses on duplicate samples of fresh curd since it is not a homogeneous mass. Accordingly processed Cheddar cheese, which should be fairly homogeneous, was employed; the moisture and salt values obtained with the micro-procedures on 10 samples from a piece of processed cheese and also the values obtained with the macro-procedures on two samples are shown (Table 1).

With the micro-procedures the values obtained for the moisture content or the salt content agreed rather closely and they also were in good agreement with the values obtained by the macro-procedures. The micro-procedures gave moisture values on the 10 samples which varied from 39.2 to 40.6 per cent and salt values on nine samples which varied from 1.97 to 2.04 per cent. The salt value on one sample was 1.93 per cent; since a small portion of this sample was difficult to ash and heating was continued for an excessive period, some of the chlorides may have been volatized. With the macro-procedures the moisture values on duplicate samples were 40.8 and 40.7 per cent and the salt values were 2.04 and 2.03 per cent.

MOISTURE AND SALT CONTENTS AND pH VALUES AT SURFACES AND CENTERS OF PIECES OF CURD

Moisture and salt contents and pH values were determined at intervals on the centers and surfaces of pieces of curd from a number of cheese; data on two representative cheese are shown (Figs. 1 and 2).

RESULTS ON CHEESE A (FIG. 1)

Moisture: At hooping, which was about one hour after the beginning of the salting, the moisture contents at both the surfaces and centers of the pieces of curd were relatively high, being 41.3 and 38.8 per cent, respectively. After pressing for five hours the moisture contents had decreased considerably at both the surfaces and centers, being 35.1 and 35.3 per cent, respectively. After pressing for 15 hours the moisture content at the surfaces was essentially the same as after five hours, being 35.0 per cent; while at the centers the moisture content was much lower, being 31.6 per cent. After pressing for 24 hours the moisture contents at the surfaces and centers were essentially the same, the values being 34.7 and 34.6 per cent, respectively. No further changes of significance occurred in the moisture contents at either the surfaces or centers.

TABLE 1

Moisture and Salt Values Obtained on Processed Cheddar Cheese With Micro- and Macro-Procedures

Micro-sample	Weight of sample	Moisture	Salt
	mg.	*pct.*	*pct.*
1	4.215	39.6	1.99
2	2.412	39.5	2.02
3[1]	2.964	39.7	1.93
4	3.251	40.0	2.02
5	3.863	40.0	2.02
6	2.338	40.6	2.04
7	4.466	39.9	2.03
8	3.208	39.6	2.03
9	2.566	39.7	2.02
10	3.463	39.2	1.97
Macro-sample			
1	.....	40.8	2.04
2	.....	40.7	2.03

[1] Heated for an excessive period.

Salt: At hooping the salt content at the surfaces was relatively high, being 2.30 per cent, while at the centers it was only 0.13 per cent. After pressing for five hours the salt content at the surfaces had decreased to 1.52 per cent and at the centers it had increased to 0.56 per cent. After pressing for 15 hours the salt content at the surfaces was essentially the same as after five hours, being 1.65 per cent, but at the centers it had increased conspicuously to 1.42 per cent. After pressing for 24 hours the salt contents at both the surfaces and centers had decreased slightly, being 1.32 and 1.15 per cent, respectively. After 24 hours no significant changes in the salt contents were noted at either the surfaces or centers.

pH: Immediately after milling the pH values at the surfaces and the centers of the unsalted pieces of curd were the same. At hooping the pH value at the surfaces had increased slightly, being 5.23, whereas at the centers it had decreased to 5.05. After pressing for five hours the pH at the surfaces had decreased slightly to 5.20, while at the centers it had decreased to 4.99. After pressing for 15 hours the pH values at both the

surfaces and centers had again decreased, the greatest decrease occurring at the surfaces where the value was 5.05 compared with 4.94 at the centers. After pressing for 24 and 48 hours the pH values at both the surfaces and centers showed further decreases; the greatest decrease occurred at the surfaces. The pH values at the surfaces after 24 and 48 hours were 5.02 and 4.91, respectively, and at the centers they were 4.91 and 4.87, respectively. After 72 hours no changes were noted in the pH values at either the surfaces or centers.

RESULTS ON CHEESE B (FIG. 2)

The same general changes in moisture and salt contents and pH values as in Fig. 1 are noticeable, although there were some minor variations.

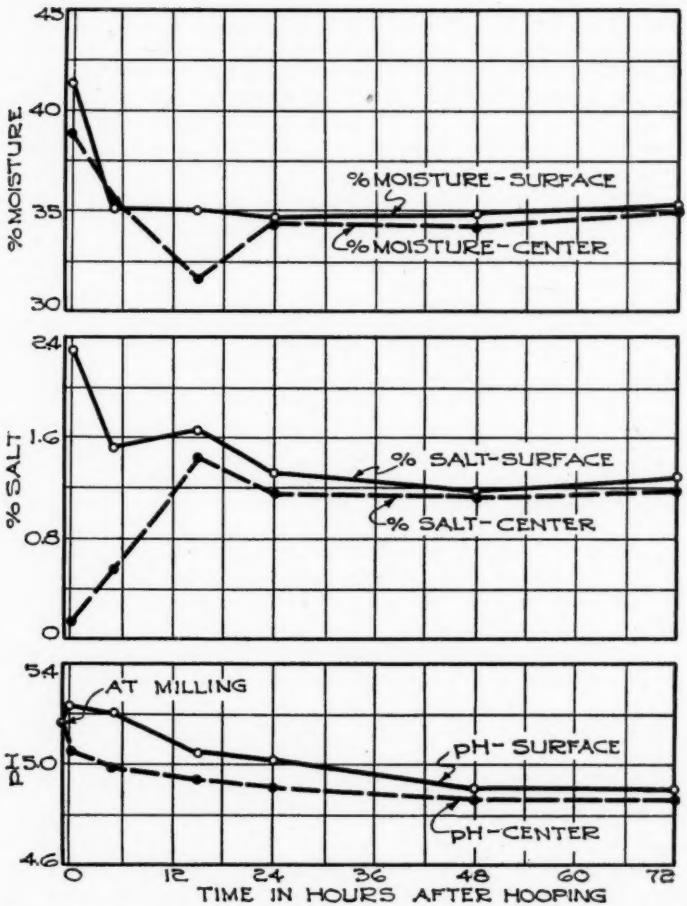

FIG. 1. Moisture and salt contents and pH values at surfaces and centers of pieces of curd taken from Cheese A at intervals after hooping.

Moisture: The moisture content of the curd was comparatively high at hooping. Large decreases occurred at both the surfaces and centers of the pieces of curd during the first 15 hours of pressing, with the greatest loss occurring at the surfaces during the first five hours. The moisture contents were appreciably higher at the surfaces than at the centers during the entire observation period.

Salt: At hooping the salt content at the surfaces was relatively high, while at the centers it was very low, the values being 2.56 and 0.10 per cent, respectively. During the early part of pressing the salt content at the surfaces decreased rapidly, whereas at the centers it increased con-

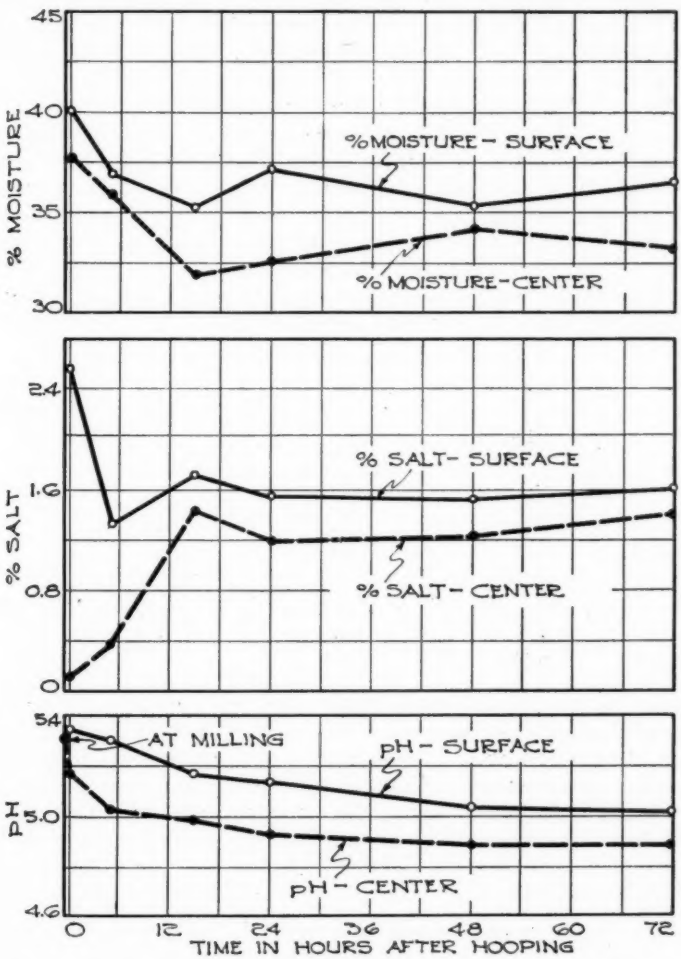

FIG. 2. Moisture and salt contents and pH values at surfaces and centers of pieces of curd taken from Cheese B at intervals after hooping.

siderably. After pressing for 15 hours the salt contents at the surfaces and centers were 1.73 and 1.41 per cent, respectively. During the latter part of the observation period only small changes occurred in the salt content at either the surfaces or centers. As with the moisture content, the salt content was higher at the surfaces than at the centers during the entire observation period.

pH: Immediately after milling the pH values were the same at the surfaces and centers of the pieces of unsalted curd. At hooping the value at the surfaces had increased slightly, whereas at the centers it had decreased considerably. During the first 15 to 24 hours in the press, the pH values decreased rather conspicuously at both the surfaces and centers, with the decrease being greater at the centers than at the surfaces during the first five hours. Late in the pressing the pH values decreased much more slowly.

EFFECT OF SALT ON PH OF CHEESE CURD

The slight increases in the pH value at the surfaces of the pieces of salted curd appeared to be rather unusual and accordingly the effect of salt on the pH of curd was investigated. A quantity of fresh, unsalted cheese curd was ground in a food chopper and the ground curd was thoroughly mixed. Weighed portions were transferred to a mortar and various amounts of salt were added to each, after which the curd and salt were thoroughly ground together and a sample removed for pH determinations; the pH values obtained are given (Table 2).

TABLE 2

Effect of Salt on pH of Unsalted Cheese Curd

Salt added to curd	pH of curd	
	Trial 1	Trial 2
pct.		
0.00	4.98	5.24
0.20	5.01	.....
0.25	.....	5.27
0.50	5.01	5.28
1.00	5.03	5.28
2.00	5.04	5.28
4.00	5.03	5.27
6.00	5.04	.....

From the data it is evident that, with the method used for determining pH, addition of salt to fresh cheese curd resulted in slightly higher values. The effect on the pH apparently was about as great with addition of relatively small amounts of salt as with addition of larger amounts. In Trials 1 and 2 the pH values on the unsalted curd were 4.98 and 5.24, respectively; after addition of 0.5 per cent salt the values were 5.01 and 5.28, respectively, and after addition of 1.0 per cent salt they were 5.03 and 5.28, respectively.

MOISTURE AND SALT DISTRIBUTION IN CHEESE RIPENED FOUR MONTHS

Since the diffusion of the moisture and salt undoubtedly continues long after the pieces of curd lose their identity, the distribution of moisture

and salt in two cheese ripened four months was investigated by analyzing 10 micro-samples picked at random from each cheese. The data (Table 3) include results of macro-determinations.

The moisture and salt distribution in each cheese was fairly uniform. With Cheese A the moisture contents of the 10 samples varied from 33.1 to 35.4 per cent and the salt contents from 1.69 to 1.80 per cent. With Cheese B the moisture and salt contents of the 10 samples varied from 33.3 to 35.4 per cent and 1.51 to 1.64 per cent, respectively. The values obtained with the micro-procedures were in general agreement with the macro-values.

TABLE 3

Moisture and Salt Distribution in Cheddar Cheese Ripened Four Months

Micro-sample	Cheese A			Cheese B		
	Weight of sample	Moisture	Salt	Weight of sample	Moisture	Salt
	mg.	*pct.*	*pct.*	*mg.*	*pct.*	*pct.*
1	1.543	33.2	1.80	2.995	35.3	1.64
2	3.658	35.4	1.79	7.379	35.4	1.55
3	4.460	34.1	1.76	3.212	34.1	1.64
4	3.264	33.2	1.69	3.095	34.3	1.56
5	3.115	34.6	1.76	5.908	33.9	1.61
6	6.019	34.2	1.70	2.759	34.7	1.64
7	2.916	33.7	1.80	2.038	33.7	1.62
8	4.277	33.8	1.74	3.920	33.3	1.57
9	3.657	34.3	1.77	3.909	34.0	1.60
10	3.347	33.1	1.69	2.626	34.4	1.51
Macro-sample						
1	.....	34.5	1.72	.....	34.6	1.72
2	.....	33.8	1.72	.....	34.3	1.73

BACTERIAL CONTENTS AT SURFACES AND CENTERS OF PIECES OF CURD

Bacterial counts were made on samples from the surfaces and centers of pieces of curd immediately after milling and also 0, 5, 15, 24, 48, and 72 hours after hooping. Because of irregularities in the data, definite changes in the bacterial counts were not evident. However, the general results indicated that immediately after milling the numbers of bacteria at the surfaces and centers of the pieces of unsalted curd were essentially the same. After salting, the number at the surfaces commonly tended to decrease during the entire observation period, whereas the number at the centers commonly showed increases for a time, after which decreases often occurred during the remainder of the observation period.

DISCUSSION

The accuracy obtained in determining the moisture and salt contents of micro-samples of Cheddar cheese was such that the micro-procedures should be useful in studying various problems, including salt distribution in cheese and its relationship to bacterial action. The procedures are not applicable to routine analyses because of the complicated technics.

The general differences in the moisture and salt contents at the surfaces and centers of the pieces of cheese curd and the changes in the contents during the pressing are clearly shown by the studies. Minor variations in the data are to be expected, since different pieces of curd were analyzed at the various intervals and some variation in the moisture and salt contents of the different pieces would be expected. Variations in the salt contents of different pieces of curd during pressing are due to the method of salting and to the position of the pieces in the hoop. Pieces of curd removed from the upper portion of a hoop may contain less moisture and salt than pieces removed from the lower portion; with the cheese in a press the moisture and salt in solution tend to drain to the lower part of the cheese. Also, relatively large amounts of moisture are pressed out of the curd and along with it considerable amounts of salt, especially from near the surfaces.

In studying differences in bacterial action at various points in cheese curd, the measurement of some chemical change that organisms bring about in the curd probably is more satisfactory than bacterial counts. Plate counts on cheese particularly can be criticized because of the difficulty in completely dispersing the curd so that all of the organisms are liberated. In general, pH values of cheese curd should be useful criteria, since ordinarily the organisms that are predominant in fresh cheese curd are lactose fermenters and produce relatively large amounts of lactic acid; also, pH values can be determined on reasonably small samples. However, the addition of salt to fresh curd caused a slight increase in the pH. Although the increase was small, it occurred consistently enough to be of significance. The increase in the pH caused by addition of salt is difficult to explain but may be due to adsorption of ions to the proteins.

In pieces of cheese curd immediately after milling, the moisture contents, the pH values, and the numbers of bacteria should be essentially the same at the surfaces and centers. After addition of salt to the surfaces of the pieces, the moisture no longer will be uniformly distributed and the bacterial action, as shown by changes in pH and bacterial numbers, will not be the same throughout a piece of curd until the salt becomes uniformly distributed. The granular salt dissolves in the moisture on or near the surfaces of the pieces of curd and, since the moisture is the continuous phase, the salt content tends to equalize throughout the entire mass by a diffusion of the salt and moisture. The salt moves toward areas of lower salt concentrations at the centers, while the moisture moves toward areas of higher salt concentrations at the surfaces. During the interval between salting and hooping, the high salt contents at the surfaces inhibit bacterial action and may cause a decrease in the number of organisms, whereas at the centers very little salt is present and conditions are favorable for bacterial action, as indicated by the rapid decrease in pH and the probable increase in numbers of organisms. During the first five hours in the press large decreases in the salt contents at the surfaces make it possible for some bacterial action to take place there, while at the centers the salt contents still are not large enough to greatly retard bacterial action; probably during the first five hours of pressing there are the greatest differences in bacterial changes at the surfaces and centers of the

pieces of curd. Later in the pressing, continued small decreases in the salt contents at the surfaces of the pieces of curd tend to favor bacterial action, whereas increasing amounts of salt at the centers tend to inhibit bacterial action. Usually after pressing for 15 hours the centers of the pieces of curd contain enough salt to definitely retard bacterial changes.

Although the differences in the salt contents at the surfaces and centers of the pieces of curd affect the bacterial action during pressing, the differences are of temporary nature and probably are not of importance in the final ripening of the cheese. At 72 hours after hooping the differences in the moisture and salt contents and the pH values at the surfaces and centers usually are small, and a further equalization of the moisture, salt, and acid in the curd occurs early in the ripening period.

SUMMARY

With a modification of the volumetric micro-method described by Ogg *et al.* (1942) for the determination of salt, a reasonable degree of accuracy was obtained in salt determinations on Cheddar cheese. Micro-moisture determinations also were reasonably accurate.

Moisture and salt contents, pH values, and numbers of bacteria were determined at the surfaces and centers of pieces of curd removed from longhorn Cheddar cheese at intervals during 72 hours after hooping the curd; the cheese were in the press for the first 48 hours.

The moisture contents decreased rather rapidly at both the surfaces and centers of pieces of curd during the first 15 hours of pressing, with the greatest loss occurring at the surfaces during the first five hours; after 15 hours the losses in moisture usually were relatively small.

The salt contents at hooping were relatively high at the surfaces of pieces of curd, while at the centers they were comparatively low. During the early part of the pressing rapid decreases in salt contents occurred at the surfaces, whereas at the centers there were conspicuous increases. After pressing for 15 hours the centers of the pieces usually contained 1.0 to 1.4 per cent salt (average 1.4 per cent for the data in the graphs); 24 hours after removal of the cheese from the press, differences in salt contents at the surfaces and centers of pieces of curd usually were small.

The pH values at milling were essentially the same at the surfaces and centers of pieces of unsalted curd. When hooped the pH values at the surfaces had increased slightly, whereas at the centers they had decreased appreciably. During the first 15 to 24 hours in the press, the pH values decreased relatively rapidly at both the surfaces and centers, with the decreases being greater at the centers than at the surfaces during the first five hours. Late in the pressing the pH values decreased much more slowly, particularly at the centers; 24 hours after removal from the press the differences in pH values at the surfaces and centers of the pieces of curd were small.

In Cheddar cheese ripened four months, the moisture and salt distribution was fairly uniform.

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IMPORTANCE OF COPPER IN CERTAIN COLOR CHANGES IN BUTTER

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Investigations have indicated the importance of copper contamination of various dairy products, particularly from the standpoint of development of off-flavors. In the case of butter, fishiness and talloviness are specific flavor defects that apparently often are caused by such contamination.

In the studies carried out in the Research Laboratory of the Sugar Creek Creamery Company on various defects of butter, color changes which apparently were related to high copper contents have been encountered. Some of these are reported herein.

METHODS OF COPPER DETERMINATIONS

With butter, the following modification of the wet-digestion method described by Williams (1931) was used: Fifty grams of butter was weighed into a 250-ml. beaker and 15 ml. of concentrated nitric acid added. The mixture was heated with constant stirring until both the fat layer and the aqueous portion became clear. The digest was allowed to cool somewhat and extracted three times with 100-ml. portions of benzene. The material remaining after removal of the fat was transferred to a 300-ml. Kjeldahl flask for the final digestion. After adding five ml. of concentrated sulfuric acid the mixture was heated carefully until the water had been driven off. Then more heat was applied and heating was continued for about one hour, when partial digestion had taken place. At this point a few crystals of sodium nitrate were added carefully, and heating continued until the digest became clear. The heat was again increased and continued until most of the sulfuric acid had been driven off.

The digest was allowed to cool and the crystalline mass was dissolved in 10 ml. of hydrochloric acid (five parts acid to two parts water), the flask being warmed gently until solution was complete; in some of the later analyses the sulfuric acid was reduced to two ml. and this was not driven off at the end of the digestion. The material in the flask was transferred to a 50-ml. volumetric flask and an aliquot removed.

A colored copper compound was developed in the aliquot, using the sodium diethyldithiocarbamate method described by Coulson (1937). The color was extracted with carbon tetrachloride and measured in a neutral wedge photometer, equipped with a Wratten No. 62 Hg green filter, which had been standardized against known copper solutions. Some of the later determinations were made by measuring the color of the copper compound with a Coleman Universal Spectrophotometer at a wave length of 440 $m\mu$.

With parchment paper, the general procedure used was the same as with butter; the paper was digested with acid but the extraction with benzene was omitted because of the absence of fat.

RESULTS

Example 1. A one-pound print of butter having large bleached areas on the surface and a pronounced tallowy flavor and odor was sent to the laboratory for an explanation of the abnormal condition. The butter came originally from a small plant in Kentucky. The copper content of the butter was found to be 1.26 p.p.m. and, presumably, the high value was related to the defect. The pH of the butter was 5.5.

Example 2. Four quarter-pound prints of butter that were bleached on the surface were sent for examination from a plant in Arkansas. The bleached portion extended almost to the center of each print and the butter had a very strong, tallowy flavor and odor. Determinations indicated an unusually high copper content of 9.9 p.p.m. The pH of the butter was 5.65.

Example 3. Four one-pound prints of butter having bluish areas on one surface of each print were sent to the laboratory by a butter marketing organization with a request for a study of the condition. In each instance the surface involved was the one on which the ends of the wrap overlapped. During the holding of the butter in a refrigerated room the bluish areas slowly bleached and the bleached areas slowly increased in size; with the bleaching, a tallowy flavor and odor developed.

Copper determinations were made on butter from one of the prints with the following results:

Bleached portion of print	14.4 p.p.m. copper
Normal portion opposite surface showing bleaching	0.5 p.p.m. copper

From the results it is evident that the copper content of the portion of the butter showing first the blue areas and then the bleaching was strikingly higher than that of the butter which remained normal in color; this suggests that a high copper content was related to the condition. Since with the four prints the face that showed the color defect regularly was the one on which the ends of the wrap overlapped, the copper contamination may have occurred in connection with the wrapping.

Example 4. A one-pound carton of butter, consisting of four separately wrapped quarter-pound prints, was forwarded to the laboratory for examination because the parchment on each print had a brown discoloration about one inch wide extending around the print near one end and the butter under the discoloration was bleached and also showed some brown color. The bleached portion extended about 0.2 inch into a print and had a pronounced tallowy flavor and odor. The remainder of each print was normal in color and flavor. The butter came originally from a Kentucky plant and had been wrapped on a machine about three weeks before it was sent to the laboratory.

Each of two of the abnormal parchments was divided into three portions and each portion was analyzed for copper; one portion was made up of the discolored area and the others represented the center and an end, both of which were normal. Each of two normal unused machine wraps and two normal unused hand wraps also were divided and three portions analyzed (Table 1). With one of the abnormal wraps the discolored area had a copper content of 309.8 μg. copper per square inch, com-

pared with an average of 4.07 μg. for the other two analyses. With the other abnormal wrap the corresponding values were 214.5 and 2.19 μg. The normal machine wraps contained relatively little copper; one contained from 0.26 to 1.18 μg. per square inch and the other from 0.19 to 0.30 μg. A similar situation existed with the normal hand wraps; one contained from 0.16 to 1.54 μg. per square inch and the other from 0.16 to 0.52 μg.

TABLE 1

Copper Contents of Portions of Abnormal and Normal Parchment Wraps

Paper examined	Copper per square inch of wrap		
	Near end of wrap	Center of wrap	End of wrap
	μg.	μg.	μg.
Abnormal wrap from defective butter	309.8[1]	3.62	4.53
Abnormal wrap from defective butter	214.5[1]	2.38	2.01
Normal machine wrap	1.18	0.34	0.26
Normal machine wrap	0.30	0.29	0.19
Normal hand wrap	1.54	0.29	0.16
Normal hand wrap	0.16	0.33	0.52

[1] Discolored area.

The discolored portion of one of the prints of butter was carefully removed and analyzed for copper. Portions of the normal butter from the inside of the print and from the surface at the end of the print also were analyzed (Table 2). The bleached butter contained 36.29 p.p.m. of copper while the normal butter contained much smaller amounts. The data suggest that some of the copper from the discolored area of a wrap was transmitted to the butter directly beneath it.

TABLE 2

Copper Contents of Various Portions of a Bleached and Discolored Print of Butter

Source of sample	Copper
	p.p.m.
Bleached portion	36.29
Normal portion, beneath bleached portion	0.46
Normal portion, end of print	0.53

Tests Involving Addition of Copper Salts to Cream Before Churning. In connection with various studies on the effects of added copper on butter, experimental churnings were made with addition of copper, usually as cupric chloride, to cream shortly before churning. The trials involved variations in the amounts of copper, variations in the pH with the copper content remaining constant, etc.

In general, the results indicated the great effect of added copper on the flavor of butter. With the added copper, fishiness often developed, particularly when the pH was relatively low, and this commonly was followed by a tallowy flavor and odor. Without the development of fishiness, tallowiness also occurred with sufficient holding. Eventually

tallowiness was evident in the control butter (without added copper), but it regularly appeared earlier with the added copper. The effect of the added copper in hastening the appearance of the tallowy flavor was very conspicuous and definite. In some cases the butter made with copper added to the cream was bleached after extended holding at temperatures approximating 4.4°C.(40°F.). This occurred most often when considerable copper was added to the cream.

DISCUSSION

The relationship between the bleaching and the high copper contents in the commercial butter is in agreement with the general information along this line and also the results obtained on addition of cupric chloride to cream just before churning. The manner in which the bleaching spread from the surface to the interior of butter in which the copper presumably was rather uniformly distributed shows the importance of air supply in the bleaching; other factors also may be of significance in the change.

The instance in which bleaching began beneath copper-contaminated portions of the wraps (from which copper could be added to the surface of the butter) and then spread to parts of butter which presumably were not so contaminated indicates that when the bleaching is once started it can progress through butter which otherwise probably would remain normal in color.

SUMMARY

Various instances were encountered in which bleaching and tallowiness in butter were accompanied by high copper contents and apparently were related to them; in one instance there were blue areas on the butter before the bleaching began.

In one instance the high copper content in the butter apparently was due to copper-contaminated wraps which had areas of brown discoloration.

Experimental churnings made with added copper, usually as cupric chloride, showed the relationship of added copper to tallowiness in the butter and also to bleaching.

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HISTAMINE CONTENT OF UNPROCESSED AND CANNED FISH. A TENTATIVE METHOD FOR QUANTITATIVE DETERMINATION OF SPOILAGE

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It was reported in an earlier paper by Geiger, Courtney, and Schnakenberg (1944) that the muscle tissue of marine fish contains a biologically very active substance which was identified as histamine. The histamine content of the fresh fish was found to be very low, but it increases *post mortem* rapidly. The changes in the histamine content were so evident and so regular that it seemed to be promising to use the histamine content as a criterion for the freshness of fish. The following experiments were performed to ascertain whether the practical application of this method would be feasible.

EXPERIMENTAL PROCEDURE

Ten grams of muscle tissue were taken from different parts of the unprocessed or of the canned fish and finely ground in a glass mortar with purified sea sand. Fifty cubic centimeters of distilled water were added slowly and the resulting paste was quantitatively transferred into a 100-c.c. volumetric flask using for the transfer an additional 20 to 25 c.c. of distilled water. The solution was then adjusted to pH 4 to 5 using hydrochloric acid, and the flasks were heated in a boiling-water bath for about 20 minutes. After cooling, distilled water was added to the 100-c.c. mark, and the mixture was well shaken and filtered by gravity through fluted sharkskin paper. Two c.c. of the filtrate are usually enough for the assay. Prior to the bio-assaying, one to two drops of rosolic acid solution (0.1 gram in 100 c.c. of 50 per cent ethanol) were added as an indicator and the filtrate was neutralized with solid powdered sodium carbonate to a slight pink color (pH 7.2). Preliminary experiments proved that histamine added to the tissues could be satisfactorily recovered by this method.

The histamine content was assayed on the isolated guinea pig intestine, [Minard (1939) and Guggenheim (1940)] as follows: A guinea pig, starved for 24 hours, was killed by a blow on the head; the ileum was removed, placed in Ringer-Locke solution,[1] and kept in the icebox. A segment of the gut (two to three cm. long) was suspended in 20 c.c. aerated Ringer-Locke solution [temp. 37°C.(98.6°F.)] and connected with a frontal writing lever. The contractions were recorded on a smoked kymograph drum. After 30 minutes in the bath, the bio-assay was started using as standard a solution of histamine-diphosphate 0.1×10^{-6}. The filtrate of the fish extract was diluted for the assay to 1:10, 1:100, or 1:1000. We determined first the contraction of the gut after addition of 0.5 ml. of the standard solution, and after washing the bath with the Ringer-Locke solution 0.5 ml.

[1] The Ringer-Locke solution contained NaCl 0.9 per cent; KCl 0.042 per cent, $CaCl_2$ 0.024 per cent, $NaHCO_3$ 0.015 per cent, dextrose 0.1 per cent, atropin 0.001 per cent.

of the diluted test solution was added to the bath. Using different amounts of the test solution we determined the dilution which had the same activity as the standard solution. From these data the histamine content of the test solution was calculated.

The values obtained by the biological assay include also the so-called "histamine-like" substances, which, however, do not interfere with the practical application of the method.

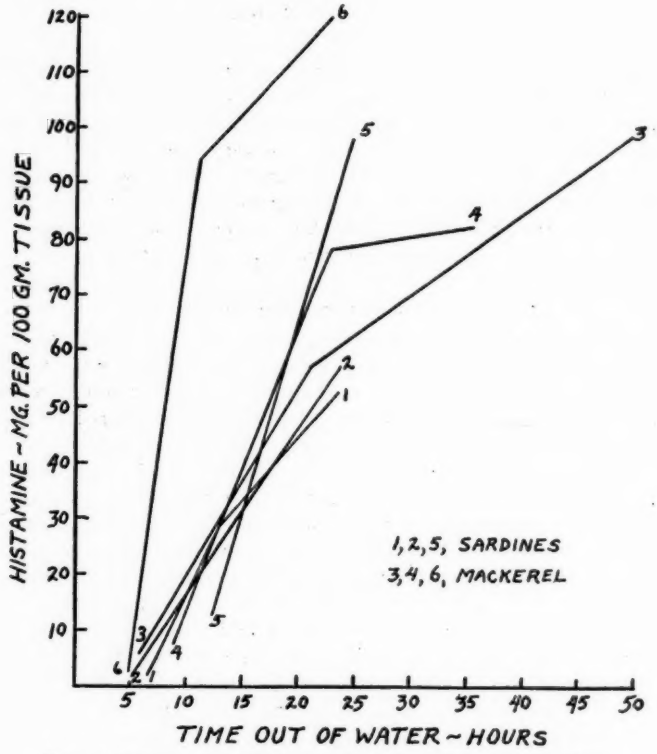

FIG. 1. Increase in histamine content with time of incubation.

EXPERIMENTAL RESULTS

We found in the experiments with California sardines (*Sardina caerulea*) and with mackerel (*Pneumatophorus jap.*) that the histamine content in the fresh fish is very low; for instance, mackerel caught alive and frozen immediately in dry ice to assure maximum freshness contains only 0.09 milligram per 100 grams of tissue. The histamine content of fresh frozen sardines was also very low, the values varying between 0.2 and 1 mg. per 100 grams of tissue.

The histamine present in the muscle tissue increases rapidly as shown (Fig. 1). The samples were kept at room temperature of 20 to 25°C. (68 to 77°F.). The histamine production seems to be the result of bac-

terial action as discussed more extensively in a previous paper by Geiger *et al.* (1944).

It was shown that canning of sardines in mustard or tomato sauce (vacuum pack) or processing of mackerel in "natural sauce" (salt solution) does not interfere with the histamine determination. We assayed the histamine content of fresh and incubated fish samples before and after canning and found, for example:

Fresh sardines before canning	4.5 mg. histamine per 100 gm. tissue
After canning in tomato sauce	3.6 mg. histamine per 100 gm. tissue
Sardines incubated for 12 hours before canning	30.0 mg. histamine per 100 gm. tissue
After canning	27.0 mg. histamine per 100 gm. tissue
Sardines incubated for 24 hours before canning	62.0 mg. histamine per 100 gm. tissue
After canning	50.0 mg. histamine per 100 gm. tissue
Fresh[2] mackerel before canning	2.5 mg. histamine per 100 gm. tissue
After canning	1.4 mg. histamine per 100 gm. tissue
Mackerel incubated for 24 hours before canning	35.0 mg. histamine per 100 gm. tissue
After canning	30.0 mg. histamine per 100 gm. tissue

Obviously the values after canning are somewhat lower. It was found that the liquids present in the can also contain some histamine, probably by diffusion from the tissues, but for practical purposes it is unnecessary to change the method or to make a correction for these losses.

The precooking of fish in steam, as in processing tuna before canning, does not change the histamine content. The histamine content of fresh albacore was found to be 2.5 mg. per 100 gm. tissue. After steaming, canning in oil, and retorting the value was 3.0 mg. per 100 gm. tissue.

The practical value of the histamine determination was demonstrated in the following experiments:

(a) In four commercial cans of tuna, we found histamine values of 1.5, 2.4, 2.8, and 3.0 mg. per 100 gm. tissue. In four experimental cans packed the same way as above but with *stale* fish, we found 48, 35, 64, and 72 mg. histamine per 100 gm. tissue.

(b) We received from the Food Distribution Administration six cans of tuna for bio-assay.[3] The histamine values of these cans were 15, 22, 18, 28, 30, and 16 mg. per 100 gm. tissue. These values are considerably higher than found in the average commercial can. None of the cans smelled strong enough for rejection. After submitting our data we were informed that the cans were packed with a definitely tainted fish. The fish lost the odor during the processing (precooking for two hours under five pounds pressure of live steam) but the histamine content still indicated the decomposed nature of the canned product.

(c) Through the courtesy of local representatives of the Food and Drug Administration we received two cans of tuna belonging to experimental packs. Can A contained according to our tests 2.4 mg. histamine and Can B contained 74.0 mg. histamine per 100 gm. tissue. The information received after bio-assaying—that Can A contained fresh tuna and

[2] "Fresh" means the condition of the fish upon arrival at the cannery, being usually four to six hours out of water.

[3] We are indebted to Mr. T. R. Hallatt for these samples.

Can B was derived from a fish already 72 hours out of water—corroborated the correctness of these values.

(d) A commercial pack of albacore contained according to our bio-assay four mg. per cent. An experimental pack of honeycombed albacore showed the high value of 170 mg. per cent. A commercial can of yellowtail contained 3.5 mg. per cent. An experimental pack of honeycombed yellowtail contained 110 mg. per cent.

(e) Two cans of sardines packed in tomato sauce were compared. Can A contained 4.5 mg. per cent, and Can B contained 37 mg. per cent histamine. Can A was a perfect can, but Can B was rejected on basis of smell test. Similarly, a can of perfect mustard sardines contained 3.5 mg. per cent in comparison with a can rejected by the organoleptic test containing 42 mg. per cent.

DISCUSSION

The methods hitherto proposed for evaluating the freshness of fish can be grouped, according to Clague (1942), into three categories: the organoleptic, the bacteriological, and the chemicophysical methods. The bio-assay method proposed above could be added as a fourth method for evaluating the relative freshness. This method seems to have the following advantages: (a) it can be learned easily even by an inexperienced technician; (b) the equipment is inexpensive, a student kymograph, some glassware, and an aquarium mist pump for aeration of the bath being all the equipment necessary to carry out the tests; (c) several tests can be performed in a relatively short time; and (d) the strips of the same guinea pig ileum can be used up to 10 days if kept at 5 to 8°C.(41 to 46.4°F.) and the Ringer-Locke solution is changed every 48 hours, Minard (1939).

As to the values ascertained by the outlined method, we find that the histamine content of fish which was not out of water more than six hours, or which was kept frozen during transport to the cannery, is usually lower than six mg. per 100 grams of tissue. Also the changes in the histamine content during the first 24 hours after catching of the fish seem to be more marked than the changes in odor, appearance, the pH, free acids, or volatile bases. Another advantage of the method seems to be that the processing of the fish, such as precooking in steam, which interferes with the subsequent determination of volatile substances, doesn't change the histamine content.

We do not feel ready to set standards for the freshness of the fish in terms of the histamine content, but on the basis of our experimental data we assume that fish containing more than 10 mg. "histamine-like" substances per 100 gm. tissue should not be regarded as "fresh."

We are quite aware that the experiments performed are so far relatively small in number, but as the results were uniform we feel justified in publishing them in order to stimulate similar experiments in other laboratories.

SUMMARY

It is proposed to use the determination of the histamine content as one criterion for the relative freshness of fish.

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RETARDATION OF FAT AUTOXIDATION IN DRIED MILKS

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The inhibition of autoxidation in fats has received much study with the view of obtaining the mechanism of both the autoxidation and its inhibition. In spite of all this work, the approach to these problems has remained for the most part empirical. However, it is known that certain polyphenols, polycarboxylic acids, and amines have the ability to retard the oxidation of fats and certain other autoxidizable substances, Bailey (1937).

Although it has been observed that milk contains certain substances which act as antioxidants to retard the onset of rancidity of milk fat very little attention has been paid to the effect of other compounds in inhibiting the oxidation of milk fat or other fats in a skim-milk medium. It has been shown that the concentration of milk by the removal of part or all of the water will increase the length of the induction period of the milk fat, Corbett and Tracy (1940) and Russell and Dahle (1943). This effect has been assigned by Corbett and Tracy (1940, 1941) to the agency of the citrates and amino acids or their esters which are found in milk. Besides these, the glucosamine of the trisaccharide attached to the casein and the phospholipids may exert some further antioxygenic influence, Lea (1939).

Since it has already been pointed out by Olcott and Mattill (1934) and György, Tomarelli, and Williamson (not yet published) that antioxidants do not behave in quite the same manner in different systems and for different fats, it was considered that the study of the effect of antioxidants on fats contained in a natural complex system, such as dried skim milk, would be of value. The large concentration of protein and carbohydrate as well as the presence of numerous other inorganic and organic compounds might be expected to have some altering effect on the inhibition of rancidity by various added antioxidants. This might occur not alone in butterfat but also in other fats in this type of system.

It was the purpose of this study to find the effect of different antioxidants on mixed animal and vegetable fats when distributed through a biochemically diverse system, such as dried skim milk.

EXPERIMENTAL PROCEDURE

From a preliminary experiment a number of substances which had proved themselves good antioxidants for a simple system, such as redistilled linoleic acid (the chief impurity being oleic acid), were chosen to be used in this experiment. Besides, a number of naturally occurring substances which were reputed to have antioxygenic potency were included, György and Tomarelli (1943). Since it has been known that thiourea will retard the oxidation of ascorbic acid, its possible capacity as a fat anti-

oxidant was considered of interest to be tested, Fearon (1942). The substances which were assayed for their antioxygenic potency and the concentrations used are listed (Table 1); the composition of the dried milks which were used to assay the antioxidants is shown (Table 2).

TABLE 1

Substances Assayed for Antioxygenic Potency in Milk Powders

No.	Antioxidants	Concentration
		pct.
1	Rice bran concentrate[1]	0.3
2	Avenex[2]	0.3
3	Mixed tocopherols (15%)[3]	0.3
4	Hydroquinone monobenzyl ether	0.3
5	4,4′-Dihydroxydiphenyl ether	0.3
6	Thiourea	0.1

[1] National Oil Products Co. [2] Quaker Oats Co. [3] Distillation Products Corp.

TABLE 2

Composition of Dried Milks Used for Testing Antioxidants

Analysis	A	B	C	D
	pct.	*pct.*	*pct.*	*pct.*
Protein	27	10	10	10
Carbohydrate	38	58	58	58
Ash	5	2	2	2
Water	2	2	2	2
Fat	28	28	28	28
Butterfat	100	....	....	50
Beef fats	....	55	55	10
Cocoanut oil	....	15	....	....
Soybean oil	....	....	45	20
Cod-liver oil	....	10	....	10
Cocoa fat	....	20	....	10

The experiment was carried out in the following manner. One hundred grams of the dried milk were thoroughly mixed with the specified amount of the substance to be assayed by grinding with a mortar and pestle. The final mixture was then placed in a brown bottle with a loosely fitting cap and incubated at 37°C.(98.6°F.) in the dark. At approximately weekly intervals five-gram samples of the mixtures were extracted and analyzed.

The extraction was carried out by mixing the five-gram aliquot with four c.c. of 95 per cent ethanol in a 50-ml. centrifuge tube until a stiff paste was formed. Then five c.c. of chloroform were added and the paste dispersed to a fine, smooth suspension. About 30 c.c. more of chloroform were added, and after thorough mixing the suspension was centrifuged. The supernatant liquid was then decanted through a filter paper into a 100-ml., round-bottom flask. The extraction was repeated. The combined extraction solvents were distilled off under reduced pressure by heating on a water bath. The extracted fats were weighed and found to be between 90 and 95 per cent of the total fat in the aliquot. The extraction procedure was found not to affect the Peroxide or Iodine Numbers of the fats since, when various rancid fats were mixed with skim-milk powder and extracted, there was no appreciable change in these values for the fats. Duplicate

samples of the extracted fat were weighed out for the analyses. The Iodine Numbers of the fats were determined by the method of Hanus. Peroxide Numbers were obtained by the method described by Lea (1939).

RESULTS AND DISCUSSION

The results of these analyses are shown (Table 3); it was judged by chemical criteria that the fats were rancid when the Peroxide Number exceeded 20 millimoles per 1000 grams. By organoleptic test, the fats usually showed an "off" odor very shortly before they were chemically rancid.

The Iodine Number of all the samples remained essentially the same throughout the experiment. The Iodine Number of the fats was followed on the presumption that it would decrease upon formation of peroxides and their subsequent decomposition. Even when the Peroxide Number rose to 134.5 millimoles the Iodine Number showed no decrease beyond that which can be accounted for by experimental error. The double bonds replaced by peroxide groups to give this Peroxide Number are relatively few as compared with the total number of double bonds. Although this Peroxide Number is indicative of a high degree of rancidity, the Iodine Number is not significantly affected.

Of all the control samples that of Fat C had the longest induction period. This extended inhibition of rancidity is probably due to the soybean oil content of this fat, since it has been shown that soybean oil contains significant quantities of tocopherols and other substances which act as strong antioxidants, Golumbic (1942). Although Fat D also contained soybean oil, it was only a minor constituent here, being less than half the concentration as found in Fat C.

The samples containing the added antioxidants had in every case, with the exception of Avenex which is doubtful, an increased induction period. Thus the previous finding of György and Tomarelli (1943), that rice bran contains a substance or substances which have antioxygenic potency, is confirmed. This also substantiates the fact that mono-ethers of hydroquinone will act as good antioxidants for fats, György, Tomarelli, and Williamson (not yet published).

It is of special interest to note that almost invariably the Peroxide Numbers of the fat samples containing thiourea were much lower than those of other samples containing other antioxidants. This is undoubtedly due to the oxidation of the small amounts of thiourea, carried over in the extraction solvents, by the iodine liberated from the KI by the fat peroxides. This was shown to be the case when different samples of the same fat containing various antioxidants, including thiourea, were analyzed for their Peroxide Numbers. In every case the Peroxide Number was the same as that of the untreated sample with the exception of the thiourea which gave a marked reduction. Quantitative titrations of an aqueous solution of thiourea with acid IBr indicated the following reaction to have taken place:

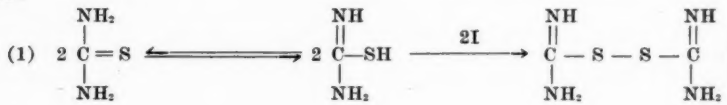


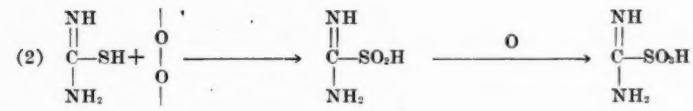
TABLE 3

Peroxide Numbers

Days	Antioxidants					
	Control	1 (Rice bran conc.)	2 (Avenex)	3 (Mixed tocopherols)	4 (Hydroxyquinone monobenzyl ether)	5 (Thiourea)
Milk Sample A						
0	4.2	.....	.....	.....	.....	.....
20	6.1	5.7	.....	.....	5.1	2.3
28	25.9	10.0	.....	.....	11.0	5.0
34	.....	10.5	.....	.....	8.9	4.7
41	.....	8.1	.....	.....	7.1	4.1
48	.....	9.5	.....	.....	7.9	3.2
55	.....	25.7	.....	.....	9.0	9.6
62	.....	.....	.....	.....	9.6	35.1
69	.....	.....	.....	.....	28.6	.....
Milk Sample B						
0	5.4	.....	.....	.....	.....	.....
5	4.0	9.4	8.4	4.7	5.1	4.0
12	8.9	10.3	6.8	7.0	4.7	1.2
19	24.9	10.1	12.1	14.6	4.2	2.5
26	36.0	10.4	7.8	11.6	5.7	3.6
33	28.6	10.0	12.1	15.7	6.3	2.4
40	134.5	16.9	12.1	20.4	6.9	6.2
47	.....	36.7	23.6	22.8	6.2	81.2
54	.....	.....	.....	.....	11.3	.....
61	.....	.....	.....	.....	13.1	.....
75	.....	.....	.....	.....	14.3	.....
82	.....	.....	.....	.....	31.7	.....

Days	Antioxidants						
	Control	1 (Rice bran conc.)	2 (Avenex)	3 (Mixed tocopherols)	4 (Hydroxyquinone monobenzyl ether)	5 (4,4'-Dihydroxydiphenyl ether)	6 (Thiourea)
Milk Sample C							
0	3.6	.....	.....	.....	.....	.....	.....
5	3.7	4.7	7.7	5.5	4.0	6.0	3.6
12	7.9	8.6	7.7	7.2	4.2	6.7	3.4
19	4.9	6.9	5.7	8.2	5.5	5.1	4.4
26	5.2	4.5	5.1	5.1	4.2	4.4	4.3
33	7.5	6.7	7.0	8.9	6.2	6.7	2.2
40	8.8	13.0	17.6	10.3	9.2	7.9	3.5
47	12.9	10.2	13.6	12.4	8.3	12.0	3.0
54	10.2	10.2	12.4	13.1	10.2	9.9	6.7
61	8.7	9.1	13.9	11.6	7.9	6.3	3.3
68	8.7	8.5	11.0	10.0	7.5	8.1	4.3
75	10.3	10.0	12.4	12.2	8.9	8.5	5.5
82	17.3	9.8	10.4	13.2	11.3	8.6	4.8
89	26.3	10.6	25.4	14.2	9.0	9.7	4.8
96	43.2	9.4	.....	11.3	.....	.....	3.8
Milk Sample D							
0	4.9	.....	.....	.....	.....	.....	.....
20	11.6	12.4	.....	.....	9.5	.....	3.8
28	26.5	11.0	.....	.....	11.0	.....	5.7
34	.....	10.5	.....	.....	8.9	.....	4.7
41	.....	9.6	.....	.....	9.7	.....	4.0
48	.....	30.0	.....	.....	9.4	.....	38.5
55	.....	.....	.....	.....	26.6	.....	.....

From this it might be considered that the thiourea would effectively mask the true Peroxide Number so that the rancidity of the fat could not be detected until long after the induction period. However, Kitamura and Suzuki (1938) have shown that thiourea will also react with peroxides to form a sulfinic acid, which in turn may be oxidized to a sulfonic acid according to the following scheme:



Thus until the fat has generated enough peroxides to completely oxidize the thiourea, it is expected that the apparent Peroxide Number will be low. At the same time, the fat peroxides will be removed by the thiourea so that rancidity will be delayed; but following the complete oxidation of the thiourea there will be a sharp rise in Peroxide Number. This is especially well illustrated in the case of Fat B where the Peroxide Number rose from 6.2 to 81.2 millimoles in a week when thiourea was the antioxidant used.

Unpublished experiments from this laboratory by György, Tomarelli, and Williamson on the inhibition of rancidity of linoleic acid by thiourea have shown this compound to be of no value as an antioxidant in a nonaqueous system. It is known that the tautomerization of thiourea to the thiol requires the presence of water, Rivier and Borrel (1928). In a nonaqueous medium the step previous to Equation 2 (shown as the first step of Equation 1) cannot take place and hence the function of thiourea as a fat antioxidant cannot be fulfilled.

The necessity for the presence of water in this reaction was tested in the following manner. To samples of a mixture of 80 per cent corn starch and 20 per cent linoleic acid (Iodine Number, 150) were added thiourea and varying amounts of water. After incubation for six days at 30°C. (86°F.) the fat was extracted with chloroform and the Iodine Number determined; results are shown (Table 4).

From the results of this experiment it is obvious that water is required to be present before thiourea can act as a fat antioxidant. In the case

TABLE 4

Effect of Water on Antioxygenic Activity of Thiourea

Sample No.	Concentration of thiourea	Concentration of water	Iodine number of linoleic acid
	pct.	*pct.*	
1	0.2	0	64.5
2	0.2	4.65	131.4
3	0.2	9.30	140.6
4	0.2	23.25	141.3
5	0	4.65	74.1
6	0	9.30	83.4
Control	0	0	70.4

of the fats in the skim-milk medium there was at least two per cent of moisture present which permitted the tautomerization of the thiourea and the subsequent reaction with the fat peroxides.

The above explanation still leaves open the problem of how the organic-solvent, insoluble thiourea comes in contact with the water-insoluble fat so that oxidation of the thiourea may take place. It seems probable that the oxidized fat molecule orients itself so that the relatively highly polar peroxide group could be immersed in the water while the rest of the molecule would remain on the other side of the fat-water interface. This would be an analogous situation to that displayed by long-chain fatty acids layered on water where the carboxyl group is dissolved in the water while the rest of the molecule is not. It would then follow that the presence of some hygroscopic substance, such as protein, would be helpful in keeping the water, containing thiourea, in intimate contact with the fat.

The author is indebted to Dr. Paul György and Dr. W. I. Patterson for their many helpful suggestions in the execution of the experiment and preparation of the manuscript.

SUMMARY

A number of antioxygenic substances were tested for their potency on different mixed fats which were incorporated into skim-milk powder. It was found that antioxidants which normally protect fat alone will also protect it in this medium. It was further observed that thiourea, which normally does not have any antioxygenic power for fats, protects the fat when combined with skim-milk powder. This is shown to be due to the presence of water. The theory of this action is discussed.

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SOME FACTORS AFFECTING THE BEHAVIOR OF CHEDDAR CHEESE IN COOKING[1]

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The qualities of a cheese that assure satisfactory performance in cooking and the best methods of cookery are not well established. The study reported here was undertaken to clarify some of the existing ideas about cheese cookery.

After a survey of a large number of cheese recipes, it appears that the practices of cheese cookery may be classified under three general methods of heat treatment: first, the melting of cheese by direct heat as in toasted sandwiches and in casseroles and other dishes having a cheese topping; second, the melting of cheese over hot water as in some cheese sauces; and third, the combination of cheese with other ingredients before heating as in cheese sauces, souffles, rarebits, etc. The majority of cooked cheese dishes belongs to the last group.

The cookery of cheese by any of these methods may present certain difficulties: the fat may separate, the cheese may become tough and stringy, or it may fail to blend satisfactorily with the other ingredients. Lowe (1943) states that an aged cheese, having considerable of its nitrogenous constituents in soluble form, tends to blend readily with other ingredients whereas an insufficiently ripened cheese may fail to combine. She recommends a temperature of 40 to 50°C. (104 to 122°F.) in preference to 60 to 70°C. (140 to 158°F.) for the blending of cheese with other ingredients and suggests that stirring aids in emulsifying the fat and so prevents its separation. Sweetman (1937) and Macleod and Nason (1937) stress the importance of low temperatures and short heating periods to avoid stringing and toughening of the heated cheese.

The effects of the composition of the cheese, the degree of ripening, the intensity and length of the heat treatment, and the reaction of the dispersing medium were investigated in this study. The soluble nitrogen was determined for many of the samples to investigate the relationship between the cooking behavior of a cheese and its content of soluble protein.

EXPERIMENTAL PROCEDURE

Samples: Six lots of Cheddar cheese were prepared by the Dairy Industry Department of the University of Wisconsin. All were made from pasteurized milk. Most of each lot of curd was pressed into lacquered cans (No. 2 size), hermetically sealed, and allowed to ripen at 7.2°C.

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(45°F.). Some of each curd was made into 20-pound daisies and paraffined. These lots included two cheese of normal composition, two of low fat content (21 and 2 per cent), one of low moisture content (36 per cent) and one of high moisture content (40 per cent). Experimental data were obtained at various intervals of storage over a period of one year.

Other cheese samples were obtained on the retail market. For these the type and approximate age were known.

Method of Sampling for Chemical Analysis: One-half of each sample of cheese was used for the "cooking tests" and the other half for chemical analysis. The latter was ground through a meat grinder four times and then transferred to tightly stoppered bottles and stored at 4.5 to 10°C. (40 to 50°F.) until tested. The storage period never exceeded 72 hours.

Acidity (pH): A Beckman pH meter, laboratory model, was used to determine the acidity of the cheese, the electrodes being inserted into a five-ml. beaker filled with the ground cheese and the pH read directly. Readings were taken on duplicate samples for each cheese.

Moisture and Fat: Moisture determinations were made according to the method recommended by Wilster, Price, Morris, Goss, and Sanders (1937). Fat was determined by the Babcock method as described by Wilster *et al.* (1937).

Nitrogen: The Kjeldahl-Gunning-Arnold method was employed for all nitrogen determinations. The data are reported as the percentage of protein in the samples. The factor 6.25 was used in calculating the percentage of protein from the percentage of nitrogen determined.

Extraction of Soluble Nitrogen Fractions: The nitrogen fractions soluble in one-per cent and five-per cent NaCl solutions were extracted from the cheese. Since it is known that the solubility of casein and paracasein in water solutions is affected by the salt content and the acidity of the solution, Sharp and McInerney (1936), it seemed desirable to control the reaction of the extracting medium. Acetate buffer solutions of pH 4.6 were used, since casein and paracasein would be expected to be least soluble at this acidity. Also 0.2 per cent $CaCl_2$ was added to the solutions so that small differences in the calcium content of the cheese samples would not affect the solubility of the cheese proteins.

An adaptation of the method of Van Slyke and Hart (1902) was used for the extractions. Ten grams of cheese and 20 grams of sea sand were ground to a homogeneous mixture by mortar and pestle. The mixture was transferred to a beaker containing 200 c.c. of buffer solution and maintained at 50 to 52°C.(122 to 125.5°F.) in a constant-temperature water bath for one hour. The mixture was agitated by means of automatic stirrers throughout the extraction. The material was then filtered through cotton and the filtrate kept in a stoppered flask under refrigeration until the nitrogen determinations were made. The period of storage did not exceed 24 hours. Nitrogen determinations were made in triplicate.

Evaluation of Cooking Quality: The methods used to evaluate the cooking quality of the cheese samples were entirely subjective. The inaccuracies of such subjective measurements are recognized but the differences were marked and it is believed that the observations are reliable.

The qualities considered in rating cooking quality included softening and melting characteristics, stringing, toughening, fat separation, and matting. The cooking quality of a cheese was rated good if the sample, when subjected to the heat treatments described below, (1) softened and melted readily; (2) remained tender; (3) either showed no tendency to string or formed very short, thin strings which broke easily; and (4) did not mat when the sample was heated in a liquid medium. Excessive fat separation was considered an undesirable quality. A progressively lower quality rating was assigned as the samples showed an increasing tendency to toughen and mat and to form thick and tenacious strings.

The following heat treatments were used in the study of cooking quality:

Direct Heat: Samples of cheese three-eighths of an inch in thickness and two inches in diameter were placed on a thin piece of wood and exposed to the direct heat of the broiling unit of an electric range. The position of the samples was such that they were exposed to a temperature of about 176.7°C.(350°F.). Samples were removed after two, two and one-half, and three minutes of exposure, and observations were made on the extent of softening and melting, length and thickness of strings, toughness, and degree of fat separation. The tests were made in duplicate.

The softening point was designated as the point at which a sample of cheese began to lose its sharp edges and to spread at the base; the melting point as the stage when the sample became semifluid in consistency and flattened, completely losing its original form.

Stringing was determined by placing a knife in the heated cheese and drawing it out slowly. The length to which the strings could be pulled before breaking and their thickness were noted. To eliminate the effect of variations in temperature, all samples were examined for stringing immediately after heating.

The resistance offered by a sample when pressed with the finger was used as an indication of toughness. Tender samples were readily depressed, while tough ones did not yield to pressure. Differences in toughness were more apparent after cooling, therefore these observations were made after the samples had cooled to room temperature.

Fat separation was measured by the relative amount of fat separating from the samples during heating.

Heating Over Hot Water: Samples of cheese three-eighths of an inch in thickness and one and one-fourth inches in diameter were placed on a shallow, flat-bottomed pan over a water bath maintained at temperatures of 75°C.(167°F.) and 100°C.(212°F.) and held at these temperatures for 10 minutes. Observations were made as for the samples exposed to direct heat. The tests were made in duplicate.

Heating in a Liquid Medium: Each cheese was heated in (1) an acetate buffer solution, pH 5.4; (2) a phosphate buffer solution, pH 6.6; and (3) distilled water. Ten-gram portions of ground cheese were added to 100 c.c. of liquid which had been heated in a water bath to temperatures of 65°C.(149°F.) and 85°C.(185°F.). The mixtures were maintained at these temperatures for 10 minutes and were stirred at two-minute intervals. Samples were then examined for matting, stringing, and toughening.

With this procedure three typical behaviors were noted: the cheese remained suspended in hard, curd-like particles; it matted forming a firm, tenacious mass; or it formed gelatinous particles that were readily dispersed in the liquid. The second type of behavior was designated as matting.

Stringing was determined by drawing a glass rod out of the mixture. If stringing occurred, the length and thickness of the strands and the ease of breaking were observed.

Finally the liquid was decanted from each mixture and the toughness of the residual cheese mass was tested. The pH of the decanted liquid was determined.

Acidity of Cheese Mixtures: Nine Cheddar cheese samples were tested in a series of buffer solutions varying in pH from 4.0 to 8.0 to further investigate the effect of pH on the behavior of heated cheese mixtures.

The procedure was the same as for the tests just described in which cheese was heated in a liquid medium.

RESULTS

Composition and Aging: A summary of the data obtained in studying the six cheese prepared by the Department of Dairy Industry is presented (Table 1). A progressive increase in the percentage of the total protein soluble in one-per cent and five-per cent NaCl solutions was found for all samples over a 12-month period of ripening. The differences noted for a given cheese sample in the percentage of the total protein which was soluble in one-per cent and in five-per cent NaCl solutions are probably within experimental error; at least these differences show no consistent trend.

TABLE 1

Composition and Cooking Quality of Six Cheddar Cheese Aged for Three, Six, and 12 Months

Lot	Type of cheese	Age	Total protein soluble in		Acidity pH	Cooking quality
			1% NaCl	5% NaCl		
		mo.	*pct.*	*pct.*		
1017 P_3.....	Skim-milk cheese	3	15.4	15.1	5.3	Very poor
		6	19.1	19.4	5.4	Very poor
		12	31.0	31.3	5.3	Very poor
1017 P_2.....	Half skim-milk cheese	3	13.4	16.1	5.3	Poor
		6	22.2	20.5	5.4	Poor
1017 P_1.....	Whole-milk cheese	3	18.2	16.1	5.3	Poor to fair
		6	22.3	21.3	5.5	Fair to good
		12	30.5	31.2	5.5	Good—
1027 P_3.....	Normal-moisture cheese	3	16.2	15.6	5.2	Poor to fair
		6	20.7	22.8	5.4	Fair to good
		12	35.5	33.9	5.4	Good—
1027 P_1.....	Low-moisture cheese	3	14.9	14.5	5.3	Poor to fair
		6	17.3	21.7	5.5	Fair
		12	29.0	28.8	5.4	Fair to good
1027 P_4.....	High-moisture cheese	3	13.7	15.3	5.3	Fair
		6	21.9	22.4	5.4	Good
		12	31.8	33.1	5.4	Good

All cheese of normal fat content exhibited a progressive improvement in cooking quality during ripening. However, the cheese of low fat content were found in all tests to form thick strings, to be very tough after heating, and to mat. These differences cannot be accounted for on the basis that a relatively small percentage of the cheese proteins existed in soluble form.

The high-moisture cheese was consistently judged somewhat superior in cooking quality to the samples of normal fat content and normal or low moisture. This was most noteworthy in the samples that had been aged for six months. The percentage of the protein in soluble form agreed closely with the values for the other samples. The low-moisture sample

showed least improvement in cooking quality at the end of a 12-month period of aging. Although the percentage of soluble protein was lower for this sample than for any of the others, this difference was not great and it is doubtful that it is significant.

The acidity (pH) of all samples fell within a narrow range, pH 5.2 to 5.4, and does not seem to account for any of the differences observed between samples.

After six and 12 months of ripening a comparison was made of the normal-fat cheese samples ripened in cans and the corresponding samples ripened in daisies. At both periods the samples ripened in cans had the higher moisture content. In general the cheese ripened in cans showed superior cooking quality at the six-month storage period but the difference was not apparent in the samples ripened for 12 months. The total protein, the percentage of protein soluble in one-per cent and five-per cent NaCl solutions, and the pH showed no consistent differences. The daisies, like the samples ripened in cans, showed a progressive increase in the percentage of the total protein that was soluble in one-per cent and five-per cent NaCl solutions with aging. There was a concurrent improvement in cooking quality. The high-moisture cheese showed the greatest improvement in cooking quality with ripening and the low-moisture cheese again exhibited the least desirable behavior.

Intensity and Length of Heat Treatment: In the tests in which cheese samples were heated over water at temperatures of 75°C.(167°F.) and 100°C.(212°F.) the samples softened more rapidly at the higher temperature. These samples also showed greater fat separation, formed longer strings, and were toughened to a greater degree as the result of heating.

Similarly the cheese samples that were exposed to direct heat, approximately 176.7°C.(350°F.), showed greatest fat separation, tended to form the longest strings, and were toughened to the greatest degree with the longest heating periods.

The temperature of heating appeared to be less important than the acidity of the mixture in determining the behavior of the cheese samples that were heated in a liquid medium. However, when matting of the samples occurred, the mats formed at 65°C.(149°F.) were less tough than those at 85°C.(185°F.). Temperature had little effect on the extent of stringing and matting. The strings did tend to be shorter and the matted cheese softer at the higher temperature but as this mixture cooled to a temperature near 65°C.(149°F.), the degree of matting and length of strings were almost identical in the samples held at the two temperatures.

Reaction of the Dispersing Medium: The behavior of a given cheese when heated in a liquid medium varied greatly with the acidity of the liquid (Fig. 1). Within the acidity range pH 4 to 8 matting of the cheese samples on heating occurred between pH 4.7 and 5.7 and again at pH 5.9. The exact pH range at which matting was observed varied for the different samples (Table 2). In general the range was narrower for the more aged cheese. In more acid solutions (below pH 4.7 to 5.0) the cheese separated from the liquid in the form of hard, curd-like particles; in less acid solutions (above pH 5.9) the samples became progressively more dispersed.

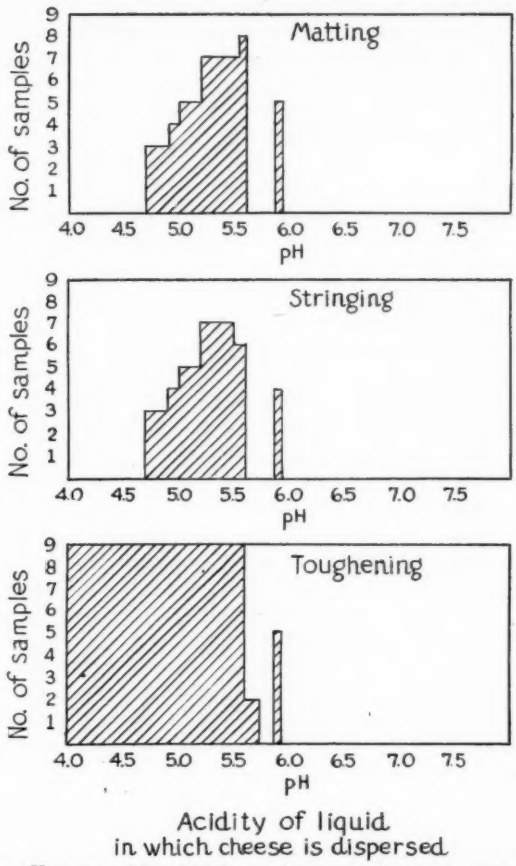

FIG. 1. The pH range within which matting, stringing, and toughening of cheese samples heated in solutions, pH 4.0 to 8.0, were observed.

TABLE 2

Effect of Acidity of Liquid in Which a Cheese Is Heated on Matting, Stringing, and Toughening of Green and Aged Cheese

Age	Number of samples	Acidity range for		
		Matting	Stringing	Toughening
mo.		*pH*	*pH*	*pH*
1- 3	5	4.7-5.6, 5.9	4.7-5.6, 5.9	4.0-5.7, 5.9
4[1]	2	5.2-5.6, 5.9	5.2-5.6, 5.9	4.0-5.6, 5.9
7-21	2	5.6, 5.9	5.9[2]	4.0-5.6, 5.9

[1] One sample, a high-moisture cheese, did not mat, string, or toughen at pH 5.9. [2] The cheese aged for 21 months did not string at any pH within the range tested.

In general the acidities at which matting occurred were also characterized by pronounced stringing of the cheese. An increase in the length and thickness of the strings appeared to parallel the extent of matting. Cheese samples heated in solutions of pH 5.9 and 5.6 and lower were tough, while in solutions of pH 5.8 and 6.0 and above, they were either soft and gelatinous or quite completely dispersed.

DISCUSSION

In general the results obtained in this study support the idea that aging improves the cooking quality of Cheddar cheese. That this improvement is related to the increase in the solubility of the cheese proteins is suggested by the fact that the two changes run more or less parallel. However, the behavior of the low-fat and of the low- and high-moisture cheese indicates that other factors are also involved.

Apparently on heating, the fat of a normal cheese softens or melts and its distribution throughout the cheese mass contributes to the consistency and tenderness of the heated product. When fat is not present, the tendency for mat formation and stringing are greatly exaggerated. It is quite possible that the role of the fat is largely a mechanical one preventing the formation of a continuous protein mass.

Several workers have shown that a high-moisture cheese ripens relatively rapidly which suggests that such cheese should show superior cooking qualities. This idea is supported by the results obtained with the high-moisture cheese and by the relatively better cooking quality of the cheese ripened in cans compared with the daisies. The differences in the amount of soluble protein as determined in these cheese were not such as to account for the differences in cooking quality observed.

Perhaps the most interesting observations made are those in relation to the importance of the acidity of the mixture in determining the behavior of cheese when heated in a liquid. The idea that the solubility of cheese proteins is largely a reflection of the acidity of the solvent is not new, but the significance of this in relation to the problems of cheese cookery has not been widely considered.

The data presented might be taken to indicate that the acidity of the medium is the most important factor in determining the behavior of a cheese when combined with other ingredients and heated. It should be pointed out, however, that all of the buffer solutions of pH 5.8 to 8.0 were phosphate buffers. It is quite possible that the ability of the phosphates to remove calcium is responsible, at least in part, for the dispersion of the cheese in these solutions. No explanation is known for the anomalous behavior of several of the samples at pH 5.9.

The results of this study suggest the possibility of improving the cooking characteristics of a cheese mixture using (1) sodium acid carbonate to reduce the acidity of the mixture or (2) a salt such as sodium citrate to remove calcium when combining cheese with other ingredients and cooking. Preliminary studies show both to be effective but, when using baking soda, the minimum amount necessary to prevent matting and stringing of a simple milk-cheese mixture unfavorably affected the flavor. The use of the citrate shows promise.

The observations on the importance of temperature in cheese cookery in general substantiate the common view that low temperatures are to be preferred. The fact that the degree of matting and stringing of cheese-liquid mixtures was less when heated at 85°C.(185°F.) than when heated at 65°C.(149°F.) was not anticipated. Since this was only a temporary difference which disappeared as the mixture was cooled, it probably has no practical significance.

SUMMARY

Six Cheddar cheese studied over a 12-month period of ripening showed a progressive increase in the amount of the total protein that was soluble in acetate buffer solutions, pH 4.6, containing one per cent and five per cent sodium chloride.

Four Cheddar cheese of normal fat content showed a progressive improvement in cooking quality over a 12-month period of ripening. This improvement was more rapid in a high-moisture cheese. In general, cheese ripened in hermetically sealed cans showed a more rapid improvement in cooking quality than part of the same curd ripened in a daisy.

There was no direct correlation between the percentage of the total protein that was soluble and the observed cooking quality. However, the cooking quality improved and the amount of soluble protein increased with the length of the ripening period.

Low-fat cheese were conspicuously poor in cooking quality.

Relatively high temperatures and long periods of heating tended to cause greater separation of fat from a cheese; matting, stringing, and toughening of the cheese mass were also increased.

The acidity and the ion content of the liquid with which a cheese is combined apparently affect the blending qualities and the tendency of the mixture to mat or string on heating. Between pH 5.2 and 5.6 the greatest tendency for matting and stringing was observed. At acidities between pH 4.0 and pH 4.7 to 5.0 the cheese separated from the liquid with which it was combined as hard, curd-like particles, while within the range pH 5.8 to 8.0 appreciable dispersion was observed. It is possible that the calcium-removing property of the phosphates in the buffer solutions of pH 5.8 and above played a major part in increasing the dispersion of the cheese.

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RIBOFLAVIN CONTENT OF MILK AND MILK PRODUCTS[1]

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Since 1937 there have been many reports in the literature giving the riboflavin content of milk and milk products. The results have varied widely. The ranges of values in some instances are for liquid whole milk, 0.6 to 3.4 μg. per ml.; dried skim milk, 13.05 to 29.4 μg. per gm.; dried whole milk, 9.3 to 29.8 μg. per gm.; and dried whey, 18.1 to 59.0 μg. per gm.

Using a fluorometric method Euler, Adler, and Schlötzer (1934) found 2.0 to 3.0 μg. of riboflavin per ml. of whole milk; Whitnah, Kunerth, and Kramer (1938) 1.16 to 1.73 μg. per gm.; Kramer, Dickman, Hildreth, Kunerth, and Riddell (1939) 2.0 μg. per gm.; and Johnson, Maynard, and Loosli (1941) 1.5 to 2.4 μg. per ml. Hand (1939) obtained a range of 1.2 to 3.4 μg. per ml. in 400 determinations. Hand and Sharp (1939) reported that 2.0 μg. per ml. was the most frequently observed value for milk from all cows.

Kunerth, Kramer, Dickman, and Whitnah (1937), with a rat-growth method of assaying for riboflavin, found values of 2.0 and 2.7 μg. per gm. of whole milk; Lunde, Kringstad, and Olsen (1939) 2.4 μg. per gm.; and Munsell (1942) 2.14 μg. per ml. Whitnah, Kunerth, and Kramer (1937) obtained 2.14 μg. per ml. by the fluorometric procedure and 2.32 μg. by a rat-growth method. Snell and Strong (1939), using the microbiological method, found whole milk to contain between 1.5 and 2.5 μg. per gm.

By means of the fluorometric procedure Hodson and Norris (1939) obtained 19.7 μg. of riboflavin per gm. of dried skim milk; Conner and Straub (1941) 19.0 to 29.0 μg. per gm.; O'Malley and Baldi (1942) 20.93 μg. per gm.; and Smith and Kuzmeski (1942) 17.7 to 21.6 μg. per gm. Wagner, Axelrod, Lipton, and Elvehjem (1940), with a rat-growth method, obtained 17.0 μg. per gm. and Sullivan and Norris (1939), with a chick assay, obtained a value of 21.2 μg. per gm. Snell and Strong (1939) and Wagner *et al.* (1940), using a microbiological analysis, obtained 17.0 μg. per gm. The same method gave Arnold, Lipsius, and Greene (1941) values of 15.9 and 16.6 μg. per gm. and Culton and Bird (1940) 21.5 and 29.4 μg. per gm.

Kemmerer (1940) presented a report of a collaborative study on the riboflavin content of dried skim milk, carried out in five laboratories using a microbiological procedure. The range of values was 14.5 to 19.0 μg. per gm. and the average was 17.4 μg. per gm.

Sullivan and Norris (1939), with a photometric method, obtained values of 29.8 and 21.4 μg. per gm. on two samples of dried whole milk. Henry, Houston, and Kon (1940), by rat-growth and fluorometric assays,

[1] The authors are indebted to Swift and Company for a grant in support of this work.

obtained 9.3 and 10.2 μg. per gm., respectively. Using a fluorometric method Smith and Kuzmeski (1942) found dried buttermilk to contain 27.3 to 30.7 μg. per gm.

Evaporated milk, analyzed by Henry *et al.* (1940) for riboflavin content by the rat-growth and fluorometric methods, was found to contain 2.6 and 2.9 μg. per gm., respectively. Murthy (1937), using a fluorometric procedure, found butter to contain 0.08 μg. per gm. and liquid buttermilk 1.55 μg. per gm. Snell and Strong (1939) reported 23.0 μg. of riboflavin per gm. of dried whey, and Hodson and Norris (1939) 21.74 μg. per gm. Liquid whey was found to contain 1.3 to 1.9 μg. per gm. by Snell and Strong (1939). Munsell (1942) reported 5.45 and 6.0 μg. per gm. for Cheddar cheese. Sharp and Hand (1940) obtained values of 8.0 μg. per gm. for American cheese, 6.0 for Swiss cheese, and 1.4 for cream cheese.

In an inclusive study of the riboflavin content of various kinds of cheese Sullivan, Bloom, and Jarmol (1943), using the microbiological method, obtained values of 2.0 μg. per gm. for cream cheese, 4.0 for Swiss cheese, and 4.1 for American cheese.

In view of the fact that many of the values for the riboflavin content of dairy products reported in the literature were obtained before the microbiological and fluorometric methods had reached a state where accurate results could be expected, and because there are many discrepancies in the values reported, an investigation of the riboflavin content of milk and milk products was undertaken. A further purpose of this study was to carry out the analyses by means of improved fluorometric and microbiological procedures, using distinctly different methods for extracting riboflavin from the samples of milk and milk products. If concurrent values were obtained by these methods, evidence would be provided that the values were representative of the actual riboflavin content. The results of the investigation are presented in this report.

EXPERIMENTAL METHODS

Eighteen different dairy products were analyzed for riboflavin content. As many samples as possible were bought on the Ithaca, N. Y., market in order to have them representative of consumer marketing conditions. When this could not be done, the samples were secured directly from the manufacturers.

The fluorometric method of Hodson and Norris (1939), as modified by Scott (1943), and a modified Snell and Strong (1939) microbiological procedure were used throughout the work. In the fluorometric method riboflavin was extracted from the samples by refluxing with an acid-acetone mixture. Filtration was carried out at a pH of 6.7 and the filtrate was acidified and treated successively with four per cent $KMnO_4$ and three per cent H_2O_2 at a pH of 3.7 to remove interfering pigments. The final filtrate was read in a Pfaltz and Bauer fluorophotometer against a standard riboflavin solution that had been subjected to the same treatment as the natural products. All readings were made at a pH of 4.

The microbiological procedure of Snell and Strong (1939) was used, but several modifications were made in the method of extracting the riboflavin. In preliminary work, autoclaving with H_2SO_4 or large volumes of

water as well as boiling with 0.1 N HCl was found to give lower results than the fluorometric method in most cases, indicating incomplete extraction. On the other hand, acid-acetone extraction with the removal of acetone by vacuum distillation or enzymatic digestion with takadiastase and papain gave concurrent results. Both these methods of extraction were satisfactory for some products, but the enzymatic digestion of Cheldelin, Eppright, Snell, and Guirard (1942) gave more consistent results for all varieties of milk products.

A further modification was made in certain cases in order to get rid of turbidity which appeared in some of the assay tubes after sterilizing and which caused high results. The fact that the presence of a precipitate causes stimulation of *Lactobacillus casei* has been reported by Bauernfeind, Sotier, and Boruff (1942) and Wegner, Kemmerer, and Fraps (1942). This condition was corrected by autoclaving the solution and filtering off the precipitate formed before placing aliquots in the assay tubes.

Since the presence of butterfat in large amounts apparently stimulated the growth of *L. casei*, all products with a fat content of five per cent or more were submitted to extraction with petroleum ether. This stimulation was no doubt similar to that reported by Strong and Carpenter (1942). With samples containing less than five per cent fat, the filtration at pH 4.5 proved to be satisfactory in removing interfering substances.

To test the validity of these two methods, recovery experiments were performed using whole-milk powder and adding amounts of synthetic riboflavin varying from five to 25 μg. per gm. of powder.

RESULTS

The results of the recovery experiments (Table 1) show the average recovery by means of the fluorometric assay to be 100.46 per cent and that by means of the microbiological assay, 99.54 per cent. These results

TABLE 1

Recovery of Synthetic Riboflavin Added to a Whole-Milk Powder

Riboflavin content	Riboflavin added	Riboflavin recovered	Recovery
μg./gm.	*μg./gm.*	*μg./gm.*	*pct.*
Fluorometric assay			
13.93	5.00	5.07	101.40
13.93	10.00	10.25	102.50
13.93	15.00	14.85	99.00
13.93	20.00	20.02	100.10
13.93	25.00	24.82	99.28
			Av. 100.46
Microbiological assay			
13.52	5.00	5.07	101.40
13.52	10.00	9.97	99.70
13.52	15.00	14.89	99.27
13.52	20.00	19.63	98.15
13.52	25.00	24.79	99.16
			Av. 99.54

demonstrate the validity of the two methods in measuring riboflavin content.

A statistical analysis of the 284 individual riboflavin values obtained in this investigation was carried out according to the method of Snedecor (1940). The results showed that there was no significant difference between the two methods used. Of the 142 different samples analyzed, 82 per cent showed less than five per cent variation in the results obtained by both methods. Therefore, since different methods of extraction as well as different methods of assay were employed, and since the values obtained by the two procedures agree, it is evident that the values given in this paper are representative of the actual values for the riboflavin content of the dairy products studied in the investigation.

TABLE 2

Riboflavin Content of Milk and Milk Products on Dry Basis

Product	Number of samples	Total solids average	Procedures		Difference between averages
			Fluorometric average	Microbiological average	
		pct.	*μg./gm.*	*μg./gm.*	*pct.*
Liquid whole milk	10	13.03	13.57	13.70	0.95
Evaporated milk					
Nonirradiated	7	27.72	12.88	12.83	0.39
Irradiated	5	26.86	13.32	13.20	0.90
Condensed milk	5	33.98	11.74	11.62	1.02
Dried skim milk					
Spray dried	11	96.88	20.68	20.22	2.22
Roller dried	11	96.61	19.70	19.24	2.34
Dried whole milk					
Spray dried	11	97.26	15.83	15.90	0.44
Roller dried	8	97.34	15.13	15.18	0.33
Dried sweet-cream buttermilk	6	96.02	34.96	35.14	0.51
Light cream	7	26.27	5.65	5.58	1.24
Cheddar cheese	18	67.20	6.97	7.04	0.99
Cream cheese	5	49.40	3.75	3.83	2.09
Cottage cheese	5	26.90	11.20	11.01	1.70
Butter	8	85.37	0.430	0.429	0.23
Liquid skim milk	8	8.62	18.71	17.97	3.96
Liquid buttermilk (cultured)	5	8.86	17.93	17.45	2.73
Dried whey	6	96.69	21.74	21.10	3.04
Liquid whey	6	6.06	20.09	20.73	3.09

The results, dry basis, of the determination of the riboflavin content of milk and milk products are given (Table 2). Dried sweet-cream buttermilk was found to be the richest source of riboflavin. No liquid churned buttermilk was available on the open market in Ithaca, but a cultured product made from pasteurized skim milk was found to contain, on the dry basis, approximately one-half as much riboflavin as dried sweet-cream buttermilk.

The riboflavin values, fresh basis, for the dairy products studied are given (Table 3); the average value for liquid whole milk was found to be 1.77 μg. per gm. This is somewhat lower than the usual average of 2.0 μg. per gm. given in the literature. Evaporated milk, when diluted with water

(1:1), is advertised to contain equivalent amounts of all the ingredients present in milk. This was found to be correct with respect to riboflavin, for the value after dilution was found to be 1.78 μg. per gm. Cheese was found to be a good source of riboflavin, Cheddar cheese containing more on the fresh basis and cottage cheese more on the dry basis.

TABLE 3

Riboflavin Content of Milk and Milk Products, Showing Range and Average on Fresh Basis

Product	Procedures			
	Fluorometric		Microbiological	
	Range	Average	Range	Average
	μg./gm.	μg./gm.	μg./gm.	μg./gm.
Liquid whole milk	1.64- 1.97	1.76	1.67- 2.01	1.78
Evaporated milk				
Nonirradiated	3.31- 3.99	3.57	3.17- 3.82	3.56
Irradiated	3.49- 3.62	3.58	3.30- 3.78	3.55
Condensed milk	3.92- 4.00	3.97	3.91- 3.97	3.93
Dried skim milk				
Spray dried	17.20-22.25	20.03	16.40-21.70	19.59
Roller dried	13.43-22.43	19.02	13.42-21.00	18.59
Dried whole milk				
Spray dried	13.57-18.60	15.40	13.93-17.85	15.47
Roller dried	12.90-17.80	14.74	13.31-16.94	14.78
Dried sweet-cream buttermilk	30.10-36.20	33.56	31.37-35.48	33.73
Light cream	1.33- 1.68	1.48	1.32- 1.62	1.46
Cheddar cheese	3.69- 5.76	4.69	3.81- 5.68	4.73
Cream cheese	1.44- 2.24	1.85	1.59- 2.19	1.89
Cottage cheese	2.45- 3.64	3.03	2.34- 3.62	2.97
Butter	0.295- 0.577	0.367	0.295- 0.572	0.366
Liquid skim milk	1.44- 1.74	1.61	1.33- 1.65	1.55
Liquid buttermilk (cultured)	1.48- 1.69	1.58	1.44- 1.64	1.54
Dried whey	18.00-23.30	21.02	18.45-22.55	20.41
Liquid whey	1.11- 1.31	1.22	1.14- 1.37	1.26

From reports in the literature the rat-assay method for determining riboflavin seems to give consistently higher results than those presented in this report. It is probable that other growth factors not in the basal diet in adequate quantities are present in the materials analyzed, with the result that somewhat higher values are obtained by the rat-assay procedure.

The riboflavin values of the dried skim milks that were analyzed were higher and the dried-whey values were somewhat lower than those previously reported. The explanation of this may be found in the fact that the riboflavin content of liquid whole milk has been found to vary with (1) the diet—Virtanen and Holmberg (1938), Hand and Sharp (1939), Kramer *et al.* (1939), and Johnson *et al.* (1941); (2) the period of lactation—Whitnah *et al.* (1938); (3) the breed of cow—Whitnah *et al.* (1938), Hand and Sharp (1939), and Kramer *et al.* (1939); and (4) the milk flow—Johnson *et al.* (1941). In confirmation of the results of Johnson *et al.*, Meade and Trone (1942) found the riboflavin content of milk to vary inversely with the milk yield. It is interesting to note in this con-

nection that the samples of dried whey were received in April when the milk flow was high and the riboflavin values low.

SUMMARY

Eighteen different dairy products have been analyzed for riboflavin content by microbiological and fluorometric procedures. A different method of extracting the riboflavin from the samples was used in each procedure. Statistical analysis based on individual values showed that there was no significant difference between these two methods when applied to dairy products. Therefore, it is believed that the values obtained represent the actual riboflavin content.

The average riboflavin values on the fresh basis of the products studied were as follows: dried sweet-cream buttermilk, 33.65; dried whey, 20.72; dried skim milk, spray process, 19.81, roller process, 18.81; dried whole milk, spray dried, 15.44, roller dried, 14.76; Cheddar cheese, 4.71; condensed milk, 3.95; evaporated milk, 3.57; cottage cheese, 3.00; cream cheese, 1.87; liquid whole milk, 1.77; liquid skim milk, 1.58; liquid buttermilk (cultured), 1.56; light cream, 1.47; liquid whey, 1.24; and butter, 0.367 μg. per gm.

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DEHYDRATION OF MEAT SCRAPPLES[1]

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Under present world conditions there is an urgent need for protein foods which are highly concentrated and which require little shipping space. Dehydrated meat is one answer to the need. Ground beef is relatively easy to dehydrate but the supply is somewhat limited owing to the long period of growth required, Kraybill (1943). Pork production could be increased in a shorter time but dehydration presents several problems.

The outstanding difficulty is the high fat content of pork as compared with beef and the greater susceptibility of pork to oxidative rancidity. This not only presents the problem of removal of excess fat previous to actual dehydration, but also makes prevention of rancidity necessary if the product is to be stored for more than a few weeks. One approach to the problem is the use of lean pork cuts, such as leg or shoulder, from which outer layers of fat can be trimmed. However, on a commercial scale it is not practical to trim carcasses to less than 20 per cent fat, which is not an adequately low level for dehydration. The method used by a large producer was that of cooking the pork, pressing out the fat and liquid, allowing the fat to coalesce in a layer and removing it by skimming, Stateler (1942). The method investigated by this laboratory was the addition of corn meal to pork in the form of scrapple. It was believed that the flavor and texture would be improved and that the corn meal would absorb excess fat and possibly act as an antioxidant. It has also been found that the use of cereals protects thiamin, according to Rice, Schultz, and Robinson (1943).

EXPERIMENTAL METHODS

Dehydration: All of the scrapples were dehydrated in a home-type, forced-draft, tunnel dehydrator, described by Cruess, Faust, and Greaves (1943). A small Thermidor combination fan and electric heater was the source of heat. No arrangement for recirculation was included. The temperature of drying varied from 48.9 to 60°C. (120 to 140°F.) as measured by thermometers placed on each tray in the dehydrator. The position of the trays was changed each hour to insure more even drying. The time required for dehydration to a moisture content of five to 10 per cent was

[1]The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the office of Scientific Research and Development and the University of California.

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seven to eight hours on the average, although this varied considerably depending on the size of the load and other factors. The tray load was about three-fourths pound per square foot.

Palatability: Palatability was judged by three persons with the exception of the tests determining the desirability of seasonings and those comparing ground pork with scrapple. The same three people judged the scrapple throughout the study. It was believed that in this way marked differences over a period of time were more likely to be noticed. The reconstituted [2] scrapples within each lot were judged and compared with the fresh fried scrapples. The points considered were flavor, texture, and appearance as affected by the degree of dehydration. In some cases the desirability of using different cereals, other meats, and antioxidants was judged.

Moisture: The majority of the determinations were made by the A.O.A.C. method (1935), drying under vacuum for about five hours at 100°C.(212°F.). The dehydrated scrapple was ground either in a corn mill or with a mortar and pestle before drying. Some determinations were made by the toluene method which was accurate within 0.5 per cent when the source of heat was carefully controlled. This method saves time only when relatively few samples are being determined, however, which was not usually true in this study.

Fat: The ground dehydrated scrapple was extracted with ethyl ether in a Soxhlet apparatus for 16 hours during the first few months of the study. Later the extraction was modified using petroleum ether and an extraction period of three hours, as described by Neustadt (1942).

DEVELOPMENT OF SCRAPPLE FORMULA

Fat Content of the Meat: Leg and shoulder cuts of pork from which the outer layers of fat were trimmed were used in this study. The fat content of approximately 20 samples chosen at random averaged 14 per cent for the fresh ground pork and varied from five to 26 per cent. The average fat content of 20 dehydrated scrapples was 27 per cent and varied from 14 to 52 per cent.

The optimum range appeared to be from 12 to 18 per cent fat in the fresh pork resulting in a fat content of 20 to 30 per cent in the dehydrated scrapple. If pork of higher fat content was used some of the fat melted out during dehydration and was lost. In addition the time required to dehydrate the scrapple to a moisture content of five to ten per cent was somewhat longer. When scrapple and ground, steamed, and raw pork made from the same lot of meat were dehydrated, the scrapple absorbed more fat than either of the ground porks and had a much better appearance. When the amount of corn meal was increased, more fat could be absorbed but the flavor of the product was much poorer. Pork of high fat content may be dehydrated as scrapple when it cannot be used as ground pork.

Effect of Varying the Water: The optimum amount of water to use in scrapple is influenced by three factors: (1) the product should be firm

[2] Reconstituted, as used in this paper, refers to the whole process of soaking and frying or cooking. Rehydrated is used for the process of soaking only.

enough to handle easily; (2) sufficient water should be present to gelatinize the corn meal; and (3) no excess water should be added, only to be removed during dehydration.

Other factors which affect the firmness of the product are the time of cooking, the grind of corn meal used, and the addition or substitution of other cereal for the corn meal.

In the standard scrapple the time of cooking was set at one-half hour; a "polenta" grind of corn meal was used and no soybean or other cereal was substituted for any of the corn meal. Under these conditions the optimum amount of water was about four times the weight of corn meal. This was determined from experiments in which the water content varied from seven to 2.5 times the weight of corn meal used.

Time of Cooking: Three different experiments were conducted to determine the best length of time for steaming the corn meal and pork together in a double boiler. The cooking times varied from one-half to two hours. Scrapple cooked for two hours did not rehydrate as well as that cooked a shorter time. There was little difference between the products cooked for one-half and for one hour. Since there was no marked improvement in texture given by the longer time of cooking, the half-hour period was selected. Less vitamin destruction would presumably occur and the time of production was materially shortened.

Effect of Grind of Corn Meal: The size of grind of corn meal was apparently the most important factor in determining the degree of rehydration of scrapple. When the ordinary corn meal sold on the retail market was used, the rehydrated scrapple tasted dry and took up only 0.5 to 0.7 milliliter of water per gram of dehydrated scrapple. When a coarser grind was used, the scrapple had a water uptake of from 1.0 to 1.7 milliliters of water per gram and tasted much better.

In the effort to find a suitable grind for optimum reconstitution, corn meal from several sources was obtained. It was found that a medium coarse grind with few fine particles was the most desirable. The so-called polenta grind filled these requirements but this varied in size considerably depending on the source. One polenta grind was too coarse resulting in a soft, tender product difficult to handle after rehydration, while another made a firm scrapple which rehydrated satisfactorily.

Since polenta grinds from different sources were not the same size, a means of describing the best grind was needed. The polenta was placed in standard sieves and shaken on a mechanical shaker for five minutes. It was determined by microscopic examination that no particles were larger than two mm., or, in miller's parlance, 16 meshes to the inch. The following table gives the percentages of particles of each size:

U. S. standard sieve number	Diameter of opening (mm.)	Per cent of corn meal retained
(Round hole)	1.0	47.1
(Round hole)	0.5	45.7
60	0.25	4.0
140	0.105	3.0
300	0.048	0.2

Effect of Varying Amount of Corn Meal: Since it was desired to have as large a proportion of pork as possible in the dehydrated product, the least amount of corn meal which could be used to produce a firm loaf of scrapple was determined. Another purpose was to determine the antioxidant effect of corn meal, which will be discussed in a later paper. Scrapples containing one-fourth, one-half, and twice the amount of corn meal and water used in the basic recipe were prepared and compared with the basic recipe. It was quite evident that the amount of corn meal could not be reduced. Increasing the amount did not appreciably improve the firmness of the loaf and the flavor was much less desirable.

Use of Seasonings: Many commercially prepared scrapples are highly seasoned and it was considered of interest to determine what kind of seasoning would be most acceptable. Eight different seasonings were used alone and in combination, in amounts just sufficient for the flavor to be definitely recognizable. The seasonings used per pound of meat were as follows: ⅛ tsp. black pepper, 1 tsp. sage, ¾ tsp. savory, ½ tsp. garlic salt, 1 tsp. onion salt, 1½ tsp. mustard, 1 tsp. smoked seasoning (Bakon yeast), and ⅜ tsp. chili powder. One combination included pepper, sage, and onion salt; the other combination included garlic salt, mustard, onion salt, and chili powder.

Ten judges tasted the scrapples after reconstitution and ranked them in the order of preference. The plain scrapple and that seasoned only with pepper ranked much higher than the others. Garlic, onion, sage, chili, and both combinations were ranked about the same, with smoked seasoning a little lower and savory considered poorest by two-thirds of the judges.

After storage for five months at 32.2°C.(90°F.) another palatability test was conducted. None of the scrapples was rancid. Nineteen judges compared seven of the reconstituted scrapples and ranked them as to desirability. The scrapple seasoned with pepper was much preferred over any other. Sage and garlic were next best, then the plain scrapple and onion. Mustard and chili pepper were the least desirable seasonings. Savory and the two combinations were omitted from this test. A possible reason for the lower rating of the plain scrapple after storage is that off-odors had developed which were masked by seasonings. These results show that scrapple seasoned with pepper and salt was preferred rather than the more highly seasoned products.

Standard Formula for Scrapple and Yield: Based on one pound of meat the following proportions have been found to be desirable:

	Metric system	English system
Ground lean pork (20% fat or less)	453 gm.	1 lb.
Water	260 ml.	1 cup
Polenta grind corn meal	67 gm.	½ cup
Salt	7 gm.	1 tsp.
Pepper	0.2 gm.	⅛ tsp.

The weighed meat was placed in the top of a double boiler and stirred frequently for about 20 minutes or until all pink color was gone. The meat was then placed over the direct flame and the water added. When

this came to the boiling point (in about two minutes), the corn meal and seasonings were added gradually with stirring. When the scrapple began to thicken after another two to three minutes, it was placed in the double boiler and steamed for 30 minutes. It was then poured into a loaf pan and cooled. The scrapple was cut into slices about $^3/_{16}$ inch thick, spread on trays, and dehydrated at 68.3°C.(155°F.) for five to six hours. The scrapple may also be poured into shallow pans to a depth of $^1/_3$ to $^1/_4$ inch and dehydrated without cooling. It should be cut in squares to prevent excessive curling during dehydration. The moisture content of an average sample was 4.4 per cent, protein content 37 per cent, and the fat content 28 per cent. The caloric value of this scrapple was calculated to be 5.03 calories per gram.

One pound of ground lean pork yielded about one and one-half pounds of fresh scrapple which dehydrated to about one-half pound. One to one and one-half pounds of reconstituted scrapple can be obtained from one-half pound of dehydrated scrapple.

The dehydrated scrapple may be reconstituted in several ways. It may be soaked in hot water for 15 minutes then fried in fat until golden brown. It may be soaked and crumbled, then used in gravy made from the soaking water as a creamed meat dish. For variation tomato sauce, onions, sage, chili powder, or other seasonings may be added. It may be soaked and then made into a loaf or patties, using eggs as a binder. The binder may be omitted if the scrapple is beaten thoroughly with an electric mixer or a whip.

Keeping Qualities: It has been conclusively demonstrated that dehydrated steamed pork turns rancid much more rapidly than dehydrated scrapple. Preliminary experiments indicate that this scrapple will not become rancid within two months when stored at 32.2°C.(90°F.) in oxygen or air. At room temperature scrapple has not become rancid for from four to nine months; and when antioxidants, such as soybeans, are used it will keep at least two months longer. Scrapple is now being stored under nitrogen at high temperatures and will undoubtedly keep for a much longer period, but how much longer is not yet definitely known. Compressed scrapple also is to be stored at high temperatures. It is believed that decreasing the air space will delay oxidative rancidity. These results will be presented in detail in a later publication.

COMPRESSION OF SCRAPPLE

Dehydrated scrapple was compressed in slices and after grinding. Pressures up to 10,000 pounds per square inch were used with and without preliminary heating, and with and without a holding period under pressure. The use of 10,000 pounds on heated scrapple produced a firm cake which would not rehydrate successfully. Even after three hours of soaking in hot water the center of such a cake was quite hard. Compression in the cold at 10,000 pounds resulted in a product that rehydrated quite well and formed a firm cake. However a pressure as great as this would probably not be practical on a commercial scale. Pressures of 1,000 to 2,000 pounds per square inch in the cold produced a crumbly cake which would not hold together. The same pressures applied to scrapple heated to 50°C.

(122°F.) held one minute produced a fairly firm cake which rehydrated readily within 20 minutes in hot water. The slices retained their shape quite well under these conditions. Merely holding under pressure without heating was not adequate for a firm cake. The reduction in volume per unit weight was 40 to 50 per cent for the ground scrapple and 50 to 70 per cent for the slices. An average sample of dehydrated scrapple reduced 60 per cent in volume contained five calories per cubic centimeter and had a density of 1.03.

To summarize, the best method to use for compressing scrapple is to heat the slices of dehydrated scrapple to 50°C. or higher, compress at 1,000 pounds per square inch and hold at that pressure one minute. The reduction in volume is about 60 per cent.

COMPARISON OF SCRAPPLE WITH GROUND DEHYDRATED PORK

Dehydrated sliced scrapple may be eaten out of hand similar to crackers. It is crisp but not hard and is not greasy. This is not true of ground dehydrated pork. When facilities for rehydration are not available, this is a distinct advantage.

Ground raw and steamed dehydrated pork and scrapple made with and without soy grits were prepared from two different lots of fresh pork. All of the products were reconstituted by soaking 20 minutes in just enough hot water to rehydrate them fully. This amount was 1.2 times the weight of the ground pork and twice the weight of the scrapples. After soaking, both were baked 10 minutes in an oven at 176.7°C.(350°F.) in the form of small loaves. The ground pork was reground before reconstitution as this had been shown previously to increase palatability.

After storage for four months at 32.2°C.(90°F.) in nitrogen, palatability tests of these dehydrated products were made by seven judges. Each judge scored the individual products on the following score card:

	Excellent	Good	Fair	Poor	Unpalatable	Comments
Flavor	40	30	20	10	0	
Texture	40	30	20	10	0	
Odor	20	15	10	5	0	

In addition, each was asked to arrange in order of preference the eight products judged. A value of 8 was assigned to the best, a value of 1 to the worst, with the other ratings in order. When two products were considered to be equally desirable they were given the average value of the ratings which they replaced.

The average palatability scores (Table 1) show that the scores of the scrapples were higher than those of the ground porks. This was particularly marked with one series of pork in which the flavor as well as texture was scored much lower. In both series texture was the characteristic of ground pork which was most objectionable. Adjectives used in describing texture were dry, sandy, and greasy. The difference between raw and steamed pork was shown only by Pork A.

Results of the preference ratings (Table 2) indicate that ground, dehydrated pork either raw or steamed was not as desirable as dehydrated

scrapple by highly significant differences in preference ratings. There was no difference in palatability between the plain and soy scrapples. The steamed dehydrated pork had a significantly higher rating than the raw dehydrated pork when both series were averaged.

TABLE 1

Average Palatability Scores of Various Forms of Dehydrated Pork

Type[1] of dehydrated pork	Flavor	Texture	Odor	Average	Acceptability
A. Ground steamed	23	20	14	57	70
Ground raw	20	14	14	48	45
Scrapple, plain	31	31	15	77	81
Scrapple with soy	29	31	15	75	75
B. Ground steamed	30	18	17	65	70
Ground raw	29	18	15	62	65
Scrapple, plain	29	32	17	78	70
Scrapple with soy	29	32	16	77	71

[1]A and B represent two different lots of pork.

TABLE 2

Comparison of Preference Ratings of Various Forms of Dehydrated Pork

Type[1] of dehydrated pork	Average rating	Standard deviation of the mean[2]
A. Ground steamed	4.5	±0.78
Ground raw	1.9	±0.37
Scrapple, plain	5.6	±0.67
Scrapple with soy grits	5.6	±0.40
B. Ground steamed	3.2	±0.59
Ground raw	2.4	±0.52
Scrapple, plain	7.4	±0.34
Scrapple with soy grits	6.1	±0.73
Average of A and B scrapple, plain	6.5	±0.43
Average of A and B scrapple with soy grits	5.9	±0.41
Average of A and B, ground steamed	3.9	±0.50
Average of A and B, both scrapples	6.2	±0.30
Average of A and B, ground raw	2.1	±0.31

[1]A and B represent two different lots of pork. [2]The difference is considered significant if it is three times the standard deviation of the difference ($\sigma_D = \sqrt{\sigma_{\bar{x}_1}^2 + \sigma_{\bar{x}_2}^2}$ where $\sigma_{\bar{x}_1}$ and $\sigma_{\bar{x}_2}$ are the standard deviation of the means).

USE OF CEREALS OTHER THAN CORN MEAL

A variety of cereals was used (1) to replace corn meal entirely and (2) to replace 25 per cent of the corn meal in scrapple since it was expected that the cereals would differ in their effect of flavor and possibly on rancidity.

The cereals used were (1) wheat germ and middlings, (2) fine cracked wheat, (3) graham flour (replacing 25 per cent of the corn meal only), (4) raw soy grits, (5) toasted soy grits, (6) chipped rye, (7) chipped barley, and (8) chipped oats. All of the cereals except the graham flour

were ground to pass through a sieve having 16 meshes to the inch, the same size found best for the corn meal.

The texture of the cooked scrapple loaves varied considerably. Of those made entirely of cereal other than corn meal only the chipped barley product was sufficiently firm. The two soy scrapples were too soft to form a loaf since starch content of soybeans is so low that gelatinization did not occur on heating. At the 25-per cent level all but the two soy scrapples were quite firm. The graham-flour loaf was dry but acceptable.

The cracked-wheat and chipped-rye scrapples had a more meaty flavor than the others. The barley scrapple also had a good flavor but those containing oats had a strong cereal flavor and were less desirable for that reason. All the others had a mild cereal flavor which was not objectionable. The flavor of the raw soy grits was much preferred over that of the toasted soy grits.

Other scrapples have been made in which 50 per cent of the corn meal was replaced by other cereals. Wheat germ and a whole-wheat cereal containing 20 per cent added germ were used primarily because of their properties as antioxidants as well as their nutritive value. They were acceptable in flavor but somewhat soft in texture. Cracked wheat and chipped rye, also prepared in 50-per cent mixtures, were firm in texture and were judged to have a better flavor than plain corn-meal scrapple.

USE OF MEATS OTHER THAN PORK

Several attempts were made to incorporate liver in scrapple. On the whole this was not successful from the standpoint of flavor. Liver, when dehydrated, developed a strong flavor which became even stronger after storage. Mixtures of pork and liver containing one-third, one-half, two-thirds, and all liver were made and various seasonings, such as onion and sage, were incorporated. None of the liver scrapples was liked by people who dislike liver. However, many of the judges who did not dislike liver considered the 50-per cent mixture seasoned with onion and sage fairly palatable.

Beef scrapple was quite successful but would offer no advantage over dehydrated beef. Scrapple made from dehydrated beef was just as acceptable as reconstituted beef scrapple. If dehydrated beef had to be kept for a considerable length of time under adverse conditions, however, the antioxidant property of the cereal might be of value.

Lamb scrapple was successful but the flavor was not as good as that of pork scrapple. Here again the antioxidant effect of the cereal might be of value.

Fish scrapple was not successful, at least when made with fillet of sole. Cooked fish developed a strong linseed-oil smell overnight and became quite unpalatable.

Chicken scrapple had little chicken flavor. The corn-meal flavor was so much stronger than that of the chicken that the product tasted like dehydrated corn-meal mush.

SUMMARY

A formula is given for making pork scrapple which will dehydrate successfully and which will not turn rancid readily. The fat content of

the fresh pork used should not be over 20 per cent, preferably 12 to 18 per cent, corresponding to a fat content in the dehydrated scrapple of about 30 per cent. The amount of water used should be as little as possible yet sufficient to hydrate the corn meal. The grind of corn meal used must be coarse enough to allow adequate rehydration of the scrapple but not so coarse as to cause excessive tenderness, and only enough corn meal must be used to bind the meat into a loaf. Salt and pepper only were used for seasoning since when other seasonings were added the flavor was not so desirable.

Scrapple may be eaten in the dehydrated form or reconstituted into patties, meat loaf, or creamed preparations. It can be compressed to about 60 per cent of its original volume to save storage and shipping space. When compressed it contains about five calories per cubic centimeter and has a density of about one gram per centimeter. An average scrapple had a moisture content of four per cent, a protein content of 37 per cent, and a fat content of 28 per cent. Palatability tests showed that dehydrated scrapple is much preferred to dehydrated ground pork, either raw or steamed.

Other cereals, particularly barley, cracked wheat, and chipped rye, may be substituted for part of the corn meal to improve the flavor. Other meats may also be substituted for pork, beef being particularly successful.

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INFLUENCE OF INGREDIENTS ON THIAMIN CONTENT OF BISCUITS

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The nation-wide enrichment of flour has aroused renewed interest in thiamin retention in baked products. One of the most common uses of flour in the home is for the making of quick breads, such as biscuits. Although some information is available regarding the pH of various biscuit mixtures and the relationship of pH to the destruction of thiamin, no work is reported showing the influence of the separate ingredients on the thiamin content of biscuits. The effect of shortening, the use of enriched flour as compared with natural high-vitamin flour, and the choice of leavening agent on the thiamin content of biscuits has been studied in the present work.

Farrer (1941) investigated the influence of pH on the destruction of thiamin at 100°C.(212°F.) and reported that destruction increased with increasing pH until 100-per cent destruction was obtained in one hour at a pH of 8. Thiamin retention in self-rising flour biscuits was studied by Barackman (1942). His findings confirmed those of Farrer, recovery of thiamin being directly proportional to the acidity of the biscuits. He also reported that 85 per cent of the thiamin survived the baking process if the pH of the baked product was 7.1 or lower. McKim and Moss (1943) observed the pH of chemically leavened products in connection with the composition of the batters and doughs, but did not investigate the thiamin retention. Many determinations have been made of actual thiamin losses in the baking of yeast bread. Hoffman, Schweitzer, and Dalby (1940) reported that losses in baking bread vary from five to nine per cent of the thiamin, and in toasting, from zero to 17 per cent. Harrel, Sherwood, Sullivan, and Rainey (1941) reported losses in thiamin content from 6.8 to 29.1 per cent between the extremes of baking times and temperatures used. According to Downs and Meckel (1943) enriched white bread toasted 50 seconds showed 13 per cent loss of thiamin. Slater and Rial (1942) determined thiamin losses in biscuits, cakes, and cereals. Schultz, Atkin, and Frey (1942) reported that the source of thiamin used in bread had no effect on the stability of the vitamin.

EXPERIMENTAL PROCEDURE

The problem of investigating the influence of ingredients on thiamin content of biscuits was organized with four main objectives: (1) to devise a method of sampling that would entail no change in the thiamin content of the baked product and would secure a homogeneous extract (since it was very likely that any demonstrable difference in thiamin content owing to choice of ingredients would be small, the necessity of avoiding losses subsequent to the baking procedure was an important consideration);

(2) to determine the effect of shortening on thiamin content by comparing the assay values of biscuits made with or without shortening; (3) to compare the effect of four chemical leavening agents on the thiamin content of biscuits and to relate thiamin content to pH of the doughs if possible; and (4) to compare the thiamin retention in biscuits made from enriched flour with that of biscuits made from a mixture of enriched flour and rice polish. The recent emphasis given rice polish as a food in the southern diet and the unsubstantiated statements frequently made in the promotion of its use have magnified the need for specific information in this regard.

The following basic recipe was used throughout the investigation:

Flour (vitamin and mineral enriched, phosphated, bleached)			200 gm.
Salt (NaCl)			4 gm.
Shortening (vegetable oil, nationally advertised brand)			25 ml.
Water tap	150 ml.	Lactic acid (2.25%)	150 ml.
Baking powder	10 gm.	or Baking soda ($NaHCO_3$)	3.5 gm.

The total quantity of enriched flour to be used for any one series of experiments was carefully blended to insure uniform distribution of synthetic thiamin before individual lots were weighed out. Dry ingredients were sifted together five times, and the dough was mixed in an electric mixer for 45 seconds. Each lot was made into 12 drop biscuits and baked on an ungreased baking sheet for 15 minutes at 218.3°C.(425°F.) in a preheated gas oven. The samples were analyzed by the Hennessy-Cerecedo thiochrome method as modified by the Research Corporation Committee on the Thiochrome Method, Hennessy (1942).

SAMPLING PROCEDURE

A satisfactory sampling method for biscuits would involve securing a representative sample of about five grams from a larger quantity of uniform, finely divided material. It has been customary to obtain such a sample by drying a rather large quantity of the product, crushing it to pass a fine sieve, mixing it, and weighing out an appropriate sample. Air-drying of biscuit samples proved completely ineffectual in the humid climate of Louisiana, and, consequently, an oven-drying procedure was the only choice left of customary methods. Haas' (1941) method calls for the drying of samples at less than 50°C.(122°F.) for not more than 24 hours. It was felt, however, that even such a low temperature as 50°C. might incur not only appreciable losses but variable ones as well because of the difficulty of securing uniform heat in the drying oven. An entirely different procedure, analogous to the sampling of vegetables for thiamin assay, was then worked out.

The biscuits were baked and allowed to cool to room temperature. An entire tray of 12 biscuits was used in obtaining a sample. The biscuits were quartered, opposite quarters retained, and cut into pieces one-eighth of an inch thick. The cut pieces were thoroughly mixed and 75 gm. weighed out on a torsion balance. Simultaneously a suitable sample was taken from the same original mixture for a dry-weight determination. The 75 gm. of cut biscuit was placed in the bowl of a Waring blendor, 200 ml. of 0.1 N H_2SO_4 added, and the mixture blended for four minutes. The blending

was stopped twice during that time to wash down the sides of the bowl with 0.1 N H_2SO_4. This mixture was transferred quantitatively to a 500-ml. volumetric flask and allowed to stand until free from air bubbles. It was then made up to volume, well mixed, poured into a large beaker, and 50-ml. aliquots were pipetted out while the mixture was kept homogeneous with an electric stirrer. The pipetting was done with a wide-tip recalibrated pipette, and uniform drainage of the pipette was observed at all times. Twenty ml. of the 0.1 N H_2SO_4 was added to each 50-ml. aliquot, and the samples were then ready for hydrolysis. Each sample thus prepared contained 7.5 gm. of moist biscuit in 70 ml. of acid, a sample comparable to five gm. of dried biscuit in 70 ml. of acid.

TABLE 1

Thiamin Content of Biscuit Samples Prepared by Blending Procedure and Drying Method

Lot	Treatment	Thiamin (gammas per gm. of dry wt.); 2 samples averaged	Increase of blended samples over dried samples
			pct.
1	Blended	2.48	6.44
	Dried	2.33	
2	Blended	2.84	0.35
	Dried	2.83	
3	Blended	2.65	16.74
	Dried	2.27	
4	Blended	2.78	7.34
	Dried	2.59	
Average	.....	.....	7.72

In substantiating the use of the blending procedure as opposed to the drying method, four lots of biscuits were baked and the thiamin-assay values so obtained were compared for the two sampling procedures. Each lot (a tray of 12 biscuits) constituted one sample and was prepared by both blending and drying. The twelve biscuits in each lot were quartered, and one-half of the quarters sampled by the drying method, the other half, by the blending procedure. These biscuits were all made with calcium-acid phosphate baking powder and the shortening was omitted, thus eliminating as many variables as possible. All four lots were baked at the same time in the same oven. The samples prepared by the Haas method were dried together for 18 hours at 45°C.(113°F.) in an Elconap drying oven. The entire quantity of crisp biscuit was then crushed to pass a fine sieve, mixed thoroughly, and a five-gm. sample weighed out. At the same time a sample was taken for dry-weight determination. The assay values for the two sampling procedures were converted to the dry-weight basis; these results are shown (Table 1). As the table indicates, the thiamin content of the blended samples was from 0.35 to 16.74 per cent higher than that

of the dried ones. This difference indicates thiamin destruction in the course of the drying method. The heat from oven drying would seem to be the logical cause of the loss. It might be well to consider, however, that failure to obtain a homogeneous sample or inefficient extraction because of the hardness of the dried biscuit, despite the fact that it had been crushed, could be factors responsible for the lower thiamin content of the dried samples. After ample trials, the blending process was selected by the authors as being the more satisfactory of the two and was used throughout the remainder of this study. A marked advantage of the blending procedure over the drying method lies in the fact that the preparation of a sample for hydrolysis is shortened by 18 to 24 hours.

INFLUENCE OF SHORTENING ON THIAMIN CONTENT

In studying the influence of shortening on thiamin content, biscuits were prepared that were identical except for the inclusion or omission of shortening. These two types of biscuits were baked together and their thiamin content compared. This first study was a preliminary experiment, and although it led to a complete investigation of the question, it contained insufficient data to be conclusive in itself. Experimental details were as follows: Enough dry ingredients for eight lots of biscuits were weighed out, mixed together thoroughly, and weighed into individual 214-gm. quantities. Calcium-acid phosphate baking powder was used. Four lots were baked at a time, two containing shortening and two without it. Positions in the baking oven were alternated for the shortened and unshortened biscuits to minimize possible variations in oven temperature. All sampling was done by blending; the calculations were made on the dry-weight basis; and all weights of the shortened samples were corrected for weight of added fat.

This preliminary study showed the unshortened samples to be 6.25 per cent higher in thiamin content than the shortened ones. As was pointed out above, later and more adequate investigation revealed these data to be incomplete, but they led to interesting developments. At the time, it was assumed that the inclusion of shortening had made the difference in thiamin content of the shortened and unshortened biscuits. During the thiamin-assay procedure, furthermore, a marked difference had been observed in the appearance of the extracts of shortened samples as compared with those of unshortened ones. The former produced an extract which was slightly cloudy in appearance and slower to pass through the base-exchange columns than were the clearer extracts of the unshortened samples. On the supposition that the cloudiness might have been due to a very fine emulsion of fat particles, and accordingly, that fat adhering to the flour particles might have interfered with the extraction of the shortened samples, it was decided to study the shortened and unshortened biscuits with the use of steapsin. Steapsin would hydrolyze the fat in the shortened samples and any difference in thiamin content of shortened and unshortened samples would not be due to fat interference. To insure the obtaining of a direct comparison, four types of samples were prepared: (1) unshortened biscuits, (2) shortened biscuits, (3) unshortened biscuits with steapsin added in the extraction process, and (4) shortened biscuits

with steapsin added in the extraction process. By adding steapsin to both shortened and unshortened biscuits two objectives would be realized: the fat of the shortened samples would be hydrolyzed and any other effects of the steapsin would appear in the unshortened biscuits as well as the shortened, thus permitting a fair comparison to be made between the two on the basis of shortening alone.

Three series of determinations (I, II, III) were made independently. The method of preparation used in the preliminary work was followed, except that alternating rows of shortened and unshortened biscuits were placed on all four baking trays, identified by marking the row position with wax pencil, and the original lots were reassembled after baking. Four aliquots were pipetted from each blended sample. Two of these were treated with steapsin and two were not, in order to obtain the comparison outlined above. Steapsin[1] (0.25 gm. per sample) was introduced along with the diastase in the sodium acetate buffer. Three independent analyses of the steapsin showed its thiamin content to be 2.69 gammas per gram, and the proper correction factor was used for the assay value of every steapsin-extracted sample before that value was recorded in Table 2. The addition of steapsin made a marked difference in the character of the extracts obtained. Extracts from both shortened and unshortened samples so treated had a sparkling clarity, filtered rapidly leaving an unusually small residue, and quickly passed through the base-exchange columns. The average time required to obtain 30 ml. of filtrate for steapsin-treated samples was 16 minutes; for untreated samples, 28 minutes; the results of Series I, II, and III appear (Table 2).

TABLE 2

Thiamin Content of Shortened, Unshortened, Steapsin Extracted-Shortened, and Steapsin Extracted-Unshortened Biscuits

Series	Number of lots averaged	Thiamin content of biscuits (gammas per gm. of dry weight) corrected for thiamin content of steapsin				Dry weight of unshortened samples	Dry weight of shortened samples
		Unshortened	Shortened	Unshortened + steapsin	Shortened + steapsin		
						pct.	*pct.*
I	2	2.20	2.36	2.31	2.46	65.7	67.1
II	2	2.45	2.27	2.76	2.45	61.8	64.0
III	2	3.59	3.68	3.80	3.84	64.5	66.0
Average	....	2.75	2.77	2.96	2.92	.....	.....

Examination of these data show that, while there may be a difference in the thiamin content of shortened and unshortened biscuits for any one series, no differences can be demonstrated when the results of a number of series are averaged. The averages (Table 2) show that the shortened biscuits exceeded the unshortened ones in thiamin content by 0.72 per cent, while the steapsin-extracted, unshortened biscuits exceeded the steapsin-extracted, shortened ones by 1.44 per cent. These percentages are within the limits of experimental error and signify no real differences. The dry-

[1] The steapsin was obtained from the Pfanstiehl Chemical Co., Waukegan, Illinois.

weight percentages for the various series have been included in Table 2 because there are indications that a relationship exists between the degree of dryness and the relative thiamin content of shortened and unshortened biscuits. When the dry-weight values exceeded 65 per cent, the shortened biscuits were higher in thiamin content than the unshortened ones, for both steapsin-treated and untreated biscuits. When the dry-weight values were below 65 per cent, the reverse was true. In the preliminary study described earlier, unshortened biscuits exceeded shortened ones in thiamin content. Here too, the dry-weight values were below 65 per cent, being 60 to 62 per cent. No attempt is made to theorize regarding the significance or insignificance of this relationship. The one conclusion reached in regard to the influence of shortening on thiamin content is that on the average no difference could be shown between the shortened and unshortened samples. There may be a relationship, however, between dryness of samples and relative thiamin content of shortened and unshortened samples for any individual set of determinations.

The increased thiamin-assay value obtained by the use of steapsin (Table 2), however, cannot be ignored. By the use of steapsin, the assay value of the unshortened samples was increased 7.6 per cent and that of the shortened samples, 5.4 per cent. Likewise, it should be pointed out that the steapsin produced this increase in every case and for all series. Since the unshortened biscuits contained inappreciable amounts of fat, there appeared to be no obvious explanation for the phenomenon. By testing an extract of the steapsin with a solution of pure starch and iodine as an indicator, however, the steapsin was revealed to contain a highly active amylase which may have been responsible for the increase obtained in the thiamin assay values of the samples as well as for the marked change in the appearance of the extracts. The higher thiamin assay values obtained by the use of steapsin indicate that the amylolytic action of Taka-diastase was insufficient to liberate all the thiamin in samples of the size used in these experiments. Qualitative tests performed with starch solutions and iodine indicator showed the starch-splitting action of the steapsin to be considerably more rapid than that of the Taka-diastase. Because of the aforementioned advantages, despite the fact that the exact nature of the action of the steapsin preparation in question is unknown, the use of such a steapsin preparation in the thiamin assay of samples similar to those analyzed in the present work is to be recommended.

INFLUENCE OF ENRICHED FLOUR AND NATURAL HIGH-VITAMIN FLOUR ON THIAMIN CONTENT

In order to compare the thiamin content of biscuits made with flour containing principally synthetic thiamin with that of biscuits made from flour containing large amounts of natural thiamin, enriched-flour biscuits and biscuits made from a combination of rice polish and enriched flour were prepared and analyzed. Because rice polish is one of the richest sources of natural thiamin known and is easily obtainable in rice-growing areas, considerable publicity has been given to its use in food preparation. Many recipes have been devised for using rice polish in baked products. The formula used in this study was taken from a series of recipes tested

and published at the Louisiana Agricultural Experiment Station.[2] Little or no information, however, has been available concerning the destruction of thiamin in rice polish during the baking process, and for this reason it was held desirable to use a flour supplemented with rice polish for this study rather than a whole-wheat flour. A ratio of three parts flour to one part rice polish by weight was used in making the rice-polish biscuits. The biscuits were made with sodium aluminum sulfate-calcium acid phosphate baking powder, and every precaution was taken to minimize any differences in oven temperature. Four lots, two of the enriched-flour biscuits and two of the rice polish-enriched flour biscuits, were baked and sampled by the blending procedure. The flour mixtures were likewise analyzed and all values converted to the dry-weight basis; steapsin was used in the analysis; the results are shown (Table 3). For all purposes

TABLE 3

Retention of Thiamin in Enriched-Flour Biscuits and Rice Polish-Enriched Flour Biscuits

Lot	Type of flour mixture	Thiamin (gammas per gm. dry wt.); average of 2	Retention	Average retention
			pct.	*pct.*
1	Enriched flour	3.64	82.16	.....
2	Enriched flour	3.60	81.26	81.71
3	Rice polish-enriched flour	8.35	84.51	.....
4	Rice polish-enriched flour	7.83	79.25	81.88
Enriched-flour biscuit mixture, uncooked		4.43	.....	.....
Rice polish-enriched flour mixture, uncooked		9.88		.....

one may say that the percentage of retention of thiamin in enriched-flour biscuits equalled that in the rice polish-enriched flour product. These findings agree with those of Schultz, Atkin, and Frey (1942), who used synthetic thiamin, high-vitamin baker's yeast, and whole wheat as thiamin sources in yeast bread and found no differences in the percentage of retention. Although the retention is the same for the two kinds of biscuits, the rice polish-enriched flour biscuit still possesses a great advantage in that its thiamin content is 2.2 times that of the enriched-flour product.

INFLUENCE OF LEAVENING AGENT ON THIAMIN CONTENT

Three typical baking powders and an approximation of the popular soda-sour milk leavening were studied. These baking powders were (a) calcium-acid phosphate type; (b) cream of tartar type; and (c) sodium aluminum sulfate-calcium acid phosphate type. The soda-sour milk combination was simulated by using correct proportions of sodium bicarbonate and 2.25 per cent lactic acid. Sour milk was not used since it would have introduced additional variables in the experiment and made a direct

[2] "The Use of Rice Polishings in Cooking" by Martha E. Hollinger; Revised September, 1942; Nutrition Research Laboratory, Louisiana State University Agricultural Experiment Station, Baton Rouge, Louisiana.

comparison difficult. The biscuits were made with shortening so that a standard product might be secured insofar as possible. Precautions were taken to minimize differences in oven temperature, correction was made for even slight differences in percentage of fat of the different samples, the blending procedure was used in sampling, and all results were expressed on the dry-weight basis. The experiment was performed with both enriched-flour biscuits and rice polish-enriched flour biscuits. Determinations of pH were made for all samples; steapsin was used in the analysis; the findings are reported (Table 4). Although the differences

TABLE 4

Thiamin Content of Enriched-Flour Biscuits and Rice Polish-Enriched Flour Biscuits Made With Four Different Chemical Leavening Agents

Lot	Leavening agent	Thiamin (gammas per gm. dry wt.); average of 2	Rank in thiamin content	pH of dough mixture	Rank in pH
	ENRICHED-FLOUR BISCUIT SERIES				
1	Soda + lactic acid	2.55	1	6.42	4
2	Cream of tartar baking powder	2.55	1	6.50	3
3	Calcium-acid phosphate baking powder	2.48	3	6.91	2
4	Sodium aluminum sulfate-calcium acid phosphate baking powder	2.41	4	7.08	1
	RICE POLISH-ENRICHED FLOUR BISCUIT SERIES				
1	Soda + lactic acid	8.44	1	6.33	4
2	Cream of tartar baking powder	8.38	2	6.45	3
3	Calcium-acid phosphate baking powder	8.02	3	7.00	2
4	Sodium aluminum sulfate-calcium acid phosphate baking powder	7.77	4	7.10	1

among the samples are small, there appears to be a definite order in the thiamin content of the two series with respect to type of leavening used. In both series, the samples ranked from highest to lowest in thiamin content as follows: soda-lactic acid, cream of tartar, calcium-acid phosphate, and sodium aluminum sulfate-calcium acid phosphate. The inverse relationship is observed in the pH values, the sodium aluminum sulfate-calcium acid phosphate baking powder producing the highest pH and the soda-lactic acid, the lowest. McKim and Moss (1943) reported the same order of pH values for the three types of baking powders as obtained in this study, but did not report values for soda-lactic acid combinations. These findings agree also with those of Farrer (1941) and Barackman (1942), i.e., the more acid the biscuits, the greater the thiamin retention. Another point of interest lies in the fact that the soda-lactic acid combination produced a biscuit having the highest thiamin content of the group, thus showing that a properly balanced recipe using soda is no more destructive of thiamin than one using baking powder. Although Table 4 contains

values for only eight samples, the same order in the thiamin content was observed in the preliminary work of the study.

SUMMARY AND CONCLUSIONS

The thiamin content of biscuits was studied with reference to the influence of (1) the inclusion or omission of shortening (2) the use of enriched flour as compared with natural high-vitamin flour and (3) the choice of leavening agent. A method of sampling the moist biscuit was developed to avoid losses in thiamin content apparently due to oven drying. Samples prepared by the new method exceeded dried samples by 7.72 per cent in thiamin content, and 18 to 24 hours of time were saved in the preparation of samples.

No significant differences could be demonstrated in the thiamin content of shortened and unshortened biscuits. The addition of steapsin to samples in the extraction process, however, effected an increase of at least five per cent in the thiamin-assay value of both shortened and unshortened samples after a correction had been made for the thiamin content of the steapsin. In addition to a higher assay value obtained by use of the steapsin, the extracts obtained after the digestion process was clearer, filtered more rapidly, and left a smaller residue than did those from samples hydrolyzed with diastase alone.

The percentage retention of thiamin in enriched-flour biscuits was equal to that in the rice polish-enriched flour biscuits, indicating thereby that the source of the thiamin had no influence on the degree of retention.

Enriched-flour and rice polish-enriched flour biscuits made with four different leavening agents ranked in the same order when arranged according to decreasing thiamin content and increasing pH. Arranged thus, they rank: soda lactic acid, cream of tartar baking powder, calcium-acid phosphate baking powder, and sodium aluminum sulfate-calcium acid phosphate baking powder.

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EFFECT OF CERTAIN PRESERVATIVES ON DETERMINATION OF STARCH BY THE DIASTASE METHOD

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The diastase method for the determination of starch consists essentially of four steps: (1) the specific action of malt diastase in hydrolyzing starch into dextrins and maltose without acting upon the pentosans and other carbohydrates which can be converted into simple sugars by boiling with HCl; (2) the removal of the insoluble carbohydrates; (3) the hydrolysis by acid of the dextrines and maltose to dextrose; and (4) the determination of the resulting dextrose.

The sensitivity of the enzyme diastase suggests that certain food preservatives might interfere with its use in the determination of starch in food products.

That small amounts of NaCl will activate diastase was recognized by Osborne (1895). Sherman, Caldwell, and Adams (1927) showed that NaCl and KNO_3 activate malt amylase when it acts upon soluble starch. Hizume (1924), however, reports that the diastase is injured by NaCl, and that neither the saccharogenic nor dextrogenic powers were activated by NaCl or KNO_3. That the anion of an added electrolyte has a greater effect upon the decomposition than the cation is noted by Biederman and Rucha (1921) and by Hahn (1922).

EXPERIMENTAL PROCEDURE

In order to obtain a pure starch the sample of starch was washed with an alcohol-ether mixture three times and filtered; washed with distilled water three times and filtered; and finally washed three times with an alcohol-ether mixture and filtered. The product was air-dried and finally dried in a vacuum oven at 70°C.(158°F.). The starch was ground and put through a 100-mesh sieve.

The diastase solution was prepared by digesting at room temperature 10 grams of Pfanstiehl diastase U.S.P. IX in 200 c.c. of water for three hours. After filtration the solution was ready for use. Because of small amounts of reducing substances in the diastase solution, it is necessary to run a blank on each new diastase solution prepared.

Exactly three grams of purified starch were weighed out together with a given amount of a preservative and both were transferred to a beaker with 40 c.c. of water and 10 c.c. of buffer solution, consisting of 0.006 molar solution of a mixture of 99 per cent KH_2PO_4 and one per cent $NaHPO_4 \cdot 2H_2O$. The pH $\pm$ of the mixture was adjusted to a value of 4.9 with either H_3PO_4 or NaOH. The mixture was gelatinized at a low heat while being stirred constantly, and was cooled to 55°C.(131°F.) in a constant temperature bath. Twenty c.c. of diastase solution were added and the mixture was digested one hour at 55°C. An additional 20-c.c. portion of diastase was added and the combined solution was digested for

one hour. The hydrolyzed starch mixture was cooled, made up to 250 c.c., and filtered. Then 200 c.c. of the filtrate were removed to a flask, 20 c.c. of HCl solution (specific gravity 1.125) were added, and the mixture was heated two and one-half hours in a boiling-water bath.

When the flask and its contents were cool, enough NaOH solution was added to neutralize the original acid used for hydrolysis. The volume was made up to 500 c.c. and filtered. Aliquots from the starch-containing originals and from the diastase blanks were analyzed for dextrose by the official Munson-Walker method, A.O.A.C. (1940). The Folin-Wu (1929) and the Poe-Edson (1932) colorimetric methods for dextrose were tried but were rejected for use in this work for two reasons: (1) the numerous dilutions increased the experimental error; (2) the diastase extract, particularly in the presence of some of the preservatives in high concentrations, imparted a color to the solutions used in the colorimetric methods, a condition which made the matching of the unknown with the standard difficult and inaccurate.

TABLE 1

Effect of Preservatives on Action of Diastase in Determination of Starch

Preservative added	Starch recovered with each preservative						
	Sodium chloride	Potassium nitrate	Sodium bisulfite	Sodium benzoate	Sodium salicylate	Boric acid	Sodium borate
gm.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
None	100.0[1]	100.0	100.0	100.0	100.0	100.0	100.0
0.1	99.9	100.0	99.9	99.9	100.0	100.0	100.1
0.5	98.8	100.0	99.8	97.9	99.6	101.2	100.0
1.0	98.4	99.3	97.2	95.8	98.0	100.4	100.8
2.0	97.6	98.0	96.4	90.3	96.4	98.8	99.8
3.0	94.9	98.0	90.9	82.4	92.5	98.8	99.6
5.0	87.4	96.7	.....	.....	.....	.....	.....

[1] The amount of starch determined in the control was considered as 100 per cent.

Various amounts of sodium chloride, potassium nitrate, sodium bisulfite, sodium benzoate, sodium salicylate, boric acid, and sodium borate were separately used in the method. A starch blank was run with each set of experiments and, for the purpose of comparison, the amount of starch determined in the mixture, containing different amounts of a given preservative, were expressed in terms of the percentage of starch recovered. Results showing the effects of different preservatives on diastase activity are recorded (Table 1).

A study of the results recorded in the table indicates that small amounts of the different preservatives used have little effect on the accuracy of the diastase method.

The addition of sodium chloride in amounts greater than two grams diminishes somewhat the percentage of starch recovered. When five grams of sodium chloride were used, only 87.4 per cent of the starch was recovered.

Potassium nitrate had very little effect on the diastase determination. Sodium bisulfite and sodium salicylate in amounts greater than two grams had some detrimental effects.

Sodium benzoate showed the greatest detrimental effect on the recovery of starch by the diastase method. When three grams of this preserva-

tive were present, only about four-fifths of the total starch present was recovered.

Neither boric acid nor its sodium salt in amounts up to three grams showed any detrimental effect on the diastase determination.

With all the preservatives except NaCl, the amounts used represent in most instances much greater amounts than would be used in food products.

CONCLUSIONS

1. The effects of seven different preservatives on the diastase method of determining starch have been investigated.

2. The effects observed are the result of the chemical nature of the preservative rather than because of a change in pH caused by the introduction of the preservative.

3. In most instances, an increasing amount of preservative effected a lower starch recovery.

4. The amount of the decrease in the percentage of starch recovered is not significant, inasmuch as the amounts of preservative used are far in excess of those ordinarily found in food products which might be examined for starch.

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PERFORMANCE STUDIES ON HOME DEHYDRATORS[1]

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During the past year considerable interest has been shown in the use of dehydration as a method for home food preservation. Factors contributing to this interest are the victory garden program, the shortage of home-canning equipment and freezing facilities, the rationing of commercially canned and frozen fruits and vegetables, and the publicity that has been given to commercial dehydration.

Information has been made available by the Department of Agriculture, experiment stations, and other agencies on home dehydration and methods of making home dehydrators.

On July 8, 1943, the War Production Board (1943) authorized the production of a limited number of home dehydrators in order to provide additional home food-preservation facilities. Under this program the unit was defined as follows: "Domestic food dehydrator means a self-contained food dehydrating unit designed for home use consisting of a cabinet, fan or blower, a thermostat, and source of heat provided by either a heating element, light bulbs, or fuel burning device and constructed so that temperature, humidity, and air flow are controlled."

In addition to the electric or forced-draft type of home dehydrator described above "natural draft" or "top-of-stove" dehydrators and oven dehydrators or drying racks have been described by various agencies and certain types have appeared on the market. These latter two types of home dehydrators depend on sources of heat already available in the home and a natural rather than a forced-air circulation to accomplish the drying. Such units may be made at home but they have certain limitations as will be described later.

In recent months this laboratory has had occasion to test and evaluate the performance of a number of home dehydrators. An attempt was made to determine what constituted a satisfactory performance and operation in a home dehydrator and to improvise a method for evaluating different models.

It is the purpose of this paper to outline the test procedure used and to present experimental results that have been obtained. The method described, although incomplete from an engineering standpoint, is easily performed and provides the desired information.

PROCEDURE FOR TESTING HOME DEHYDRATORS

Medium-sized carrots were chosen as a test food because they are generally available and have been found to possess relatively uniform drying characteristics.

[1] Contribution No. 497, Massachusetts Agricultural Experiment Station.

The procedure as adopted for testing home dehydrators is as follows:

1. Peel medium-sized fresh carrots, slice into 3/16-inch slices with a mechanical slicer.
2. Blanch sliced carrots for 10 minutes in steam.
3. Weigh the empty and thoroughly dry dehydrator trays.
4. Load each tray with blanched carrots on basis of 1½ pounds carrots per square foot of drying area. The carrots should be evenly distributed over the trays.
5. Weigh loaded dehydrator trays.
6. Operate the dehydrator with the heat on for 15 minutes prior to the start of the test in order to warm up the cabinet. The temperature of the dehydrator should be set at 62.8-65.6°C.(145-150°F.).
7. Place the weighed trays of carrots in the dehydrator, close the door, and begin the drying operation. The total electrical energy consumed during the test should be measured by means of a watt-hour meter.
8. At hourly intervals the trays should be weighed individually and then replaced in their original position in the dehydrator (unless design of unit calls for rotating the trays). The temperature on the different trays should be measured periodically by means of a thermometer or thermocouple. When the trays are removed and weighed, the uniformity of drying over the tray area should be observed. The hourly consumption of electricity should be recorded.
9. The performance of the thermostat (if present) should be checked by measuring the temperature variation through several cycles at intervals during the test.
10. The test is considered to be completed when no weight is lost during a one-hour period.

Upon completion, this test will provide the following data regarding the performance of the dehydrator being examined:

1. *Rate of drying and drying time:* A drying curve may be constructed from the data obtained to indicate the rate of drying.
2. *Uniformity of air flow and temperature:* The variation in weight and appearance between the different trays during drying provide a good picture of the uniformity of air flow and temperature.
3. *Performance of thermostat:* The ability of the thermostat to control the temperature within a narrow range is indicated by maximum and minimum temperature variations during several cycles.
4. *Operating efficiency:* May be calculated from the amount of energy consumed and the amount of water evaporated.
5. *Quality of finished product:* The dried product should possess a good color and appearance and a natural flavor when reconstituted immediately after drying.

RELATIVE OPERATING EFFICIENCY

From the relation between the amount of heat actually used in the evaporation of water from the product and the total amount of energy utilized during the drying operation, the relative operating efficiency of a dehydrator may be calculated. In the calculations it may be assumed that one kilowatt-hour of electricity is equivalent to 3,412 B.t.u. and that 1,000 B.t.u. is the average quantity of heat required to evaporate one pound of water. The relative operating efficiency of the dehydrator can be expressed as follows:

$$\text{Relative operating efficiency} = \frac{\text{Pounds water evaporated} \times 1{,}000 \text{ B.t.u.}}{\text{Kilowatt-hours electricity used} \times 3{,}412} \times 100.$$

In home dehydrators with forced-draft circulation the relative operating efficiency takes into consideration the energy required to operate the motor and hence is not an actual evaluation of the thermal efficiency of the unit.

EXPERIMENTAL RESULTS

Experimental results obtained in testing different models of home dehydrators are present (Table 1). In the two natural-draft units and one of the forced-draft units the trays were rotated during the drying operation. With units of this type significant data on the weight variation between trays cannot be obtained because the rate of drying in the upper part of the dehydrator is slower than in the lower part. In practice this variation is overcome by rotating the trays.

TABLE 1

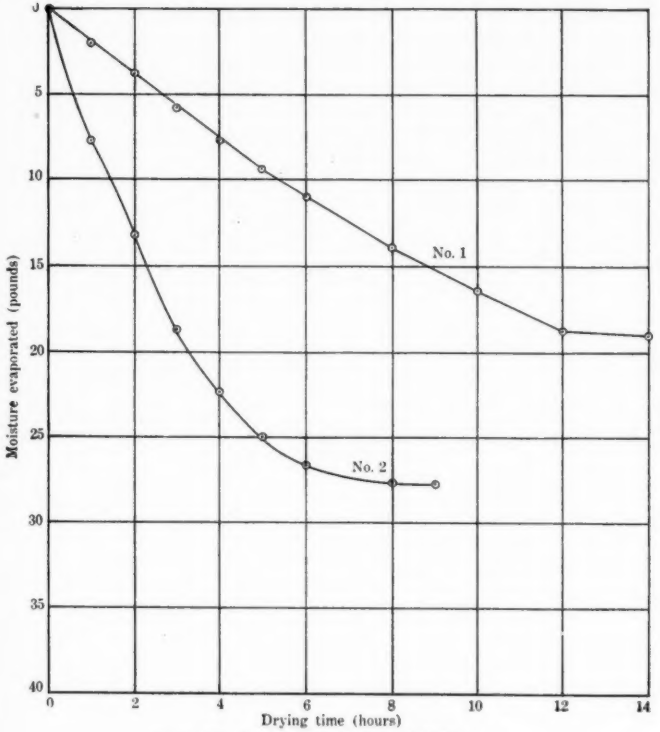
Performance Data Obtained During Dehydration of Sliced Carrots[1] *in Different Kinds of Home Dehydrators*

Model	Type	Weight of load	Amount of moisture removed	Drying time	Maximum net-weight variation between trays per sq. ft. tray area	Time when maximum variation occurred	Electricity consumed	Relative operating efficiency
		oz.	*oz.*	*hr.*	*oz.*	*hr.*	*kw.-hr.*	*pct.*
1	Forced draft	212	184	9	2.2	3	9.8	31.4
2	Forced draft	89	77	7	5.0	2	3.3	42.6
3	Forced draft	252	224	8	3.1	3	7.4	55.4
4	Forced draft	200	177	9	4.3	3	8.4	38.7
5	Forced draft	235	208	11	1.9	5	9.2	41.4
6	Forced draft	252	224	14	6.3	6	14.0	29.4
7	Forced draft	209	184	8	3.0	4	8.0	41.6
8	Forced draft	144	129	11	3.2	3	5.4	43.7
9	Forced draft	489	434	9	2.4	2	19.4	40.9
10	Forced draft	203	177	12	....	....	8.8	36.9
11	Natural draft	140	122	14	....	....	4.9	45.4
12	Natural draft	71	62.5	16	....	....	4.8	24.2
13	Natural draft	118	103	16	....	....	6.5	29.0
14	Oven	80	69	8	....	....	85.0[2]	6.3
15	Oven	34	30	5	....	....	29.0[2]	8.1

[1] 1½ pounds per square foot of tray area. [2] Cubic feet of gas.

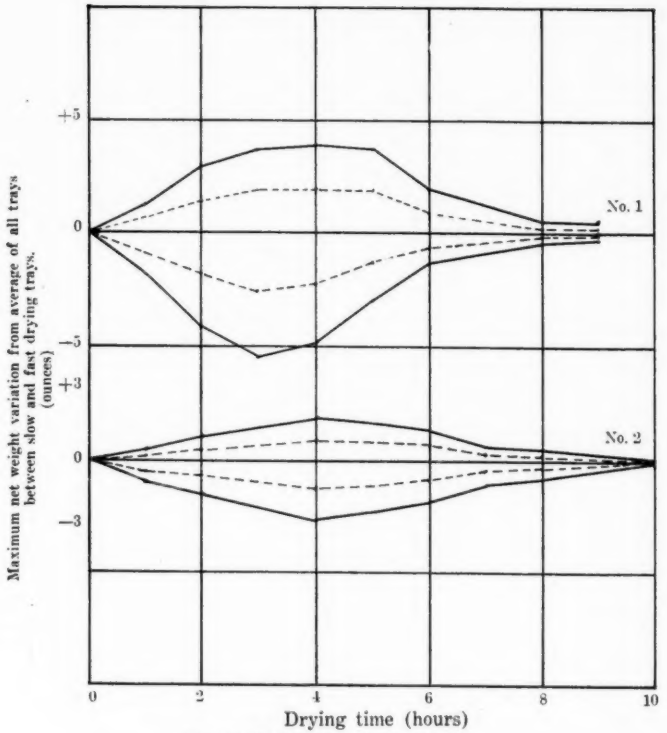
Typical drying curves representative of forced-draft and natural-draft dehydrators (Fig. 1) indicate that in the natural-draft type of dehydrator drying time is relatively long while the forced-draft type of dehydrator is characterized by a much shorter drying time in which the rate is quite rapid except near the end of the drying period.

As mentioned above a small variation in the drying rate of different trays is indicative of uniform temperature and air circulation. Data are presented to show the degree of uniformity of drying to be expected in a dehydrator of good design as contrasted with a dehydrator characterized by uneven temperatures and air flow (Fig. 2). The greatest variation in the drying rate between different trays has been found to occur when the drying was from 30 to 50 per cent complete as measured in terms of total required time. Based on present experience the maximum variation in



Curve No. 1—Natural draft dehydrator.
Curve No. 2—Forced draft dehydrator.

FIG. 1. Typical drying curves to indicate difference in rate of drying between forced-draft and natural-draft dehydrators.

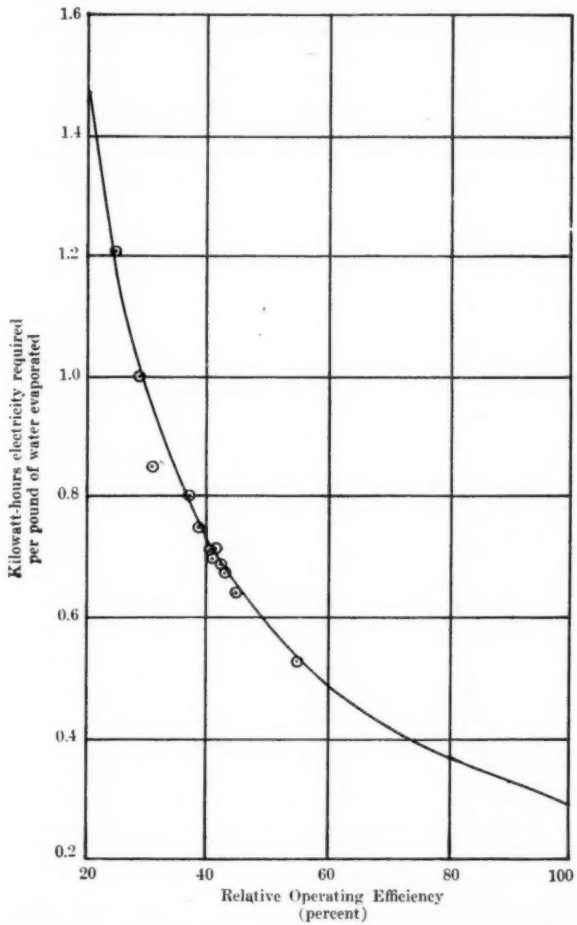


No. 1—Dehydrator of poor design.
No. 2—Dehydrator of good design.
———— Based on total tray area.
---------- Based on 1.0 sq. ft. tray area.

FIG. 2. Typical drying characteristics of good and poorly designed forced-draft home dehydrators as shown by net-weight variation between trays during the dehydration of sliced carrots. (Tray loading of 1.5 lb./sq. ft.)

weight between different trays in a satisfactory dehydrator should not exceed 3.2 ounces per square foot of tray area when loaded with 1½ pounds of sliced carrots per square foot.

The relative operating efficiency of a unit has been found to provide a good picture of its over-all performance. In general if the relative operating efficiency is 40 per cent or greater, one may expect that the dehydrator will dehydrate foods satisfactorily. The theoretical relationship between relative operating efficiency and kilowatt hours of electricity required to evaporate one pound of water is shown (Fig. 3.). In dehydrators with a



⊙ Relative operating efficiency as determined experimentally for different models of home dehydrators.

FIG. 3. Relationship between energy requirements and relative operating efficiency of home dehydrators.

forced-draft home dehydrators as shown by net-weight variation between trays during the dehydration of sliced carrots. (Tray loading of 1.5 lb./sq. ft.)

relative operating efficiency below approximately 40 per cent a marked reduction in the energy required is necessary to realize a significant increase in efficiency. Such a condition has been found to be indicative of a definitely poor design or construction. In units with a relative operating efficiency of 40 per cent or over, an increase in efficiency is accompanied by a relatively small reduction in energy requirements. Based upon present models of home dehydrators it is questionable how much their relative operating efficiency could be increased over 50 to 60 per cent. These observations are essentially in agreement with those of Cruess (1943). According to this author a well-designed commercial dehydrator should show a fuel efficiency of at least 40 per cent, and in his opinion an efficiency above 50 per cent is very difficult to obtain.

TABLE 2

Effect of Kind of Product Being Dried on Performance of a Home Dehydrator

Product	Weight of load	Moisture removed	Drying time	Electricity consumed	Relative operating efficiency
	oz.	*oz.*	*hr.*	*kw.-hr.*	*pct.*
Beans, green	173	157	7.0	6.7	43.0
Beets (strips)	272	230	12.5	10.0	42.2
Cabbage (shreds)	188	174	9.5	7.7	41.5
Carrots (slices)	209	184	8.0	8.0	41.6
Kale	64	57	2.3	2.2	47.5
Potatoes, Irish (strips)	226	187	10.0	7.4	46.3
Potatoes, sweet (slices)	320	232	13.8	13.7	31.1
Apples (⅛ sections)	288	257	17.0	11.7	40.3
Peaches (halves)	308	276	20.0	16.6	34.1
Plums, blue (halves)	398	314	24.5	19.3	30.2
Plums, red (halves)	423	355	22.0	17.8	36.7

TABLE 3

Effect of Tray Load on Drying Time and Performance of a Home Dehydrator

Tray load per square foot	Sliced carrots		Potato strips	
	Drying time	Relative operating efficiency	Drying time	Relative operating efficiency
lb.	*hr.*	*pct.*	*hr.*	*pct.*
0.5	8	18.8	7	27.4
1.0	10	33.8	9¾	37.8
1.5	12	39.4	12	40.6
2.0	16	45.2	16	45.0
3.0	22	51.5	....	....

EFFECT OF TRAY LOAD AND KIND OF PRODUCT ON PERFORMANCE OF HOME DEHYDRATORS

The operating efficiency and performance of home dehydrators is also influenced by the kind of product being dried and the tray load (Tables 2 and 3). The performance of a dehydrator improves with the speed with which a product gives up its moisture. The efficiency of operation improves with a heavier tray loading. However, this increased capacity is

achieved at the cost of an increase in drying time. This factor deserves careful consideration because, as is well known, rapid drying makes for a better finished product.

In home dehydration it is essential that the product be thoroughly dried. It is important to remember that the lower the final moisture content, the slower will be the rate of loss of quality and vitamins during storage.

PERFORMANCE REQUIREMENTS OF A SATISFACTORY HOME DEHYDRATOR

On a basis of experimental work and the standardized test outlined above, it is believed that in most cases a home dehydrator may be considered satisfactory from the performance standpoint if it meets the following requirements:

1. The dehydrator should have a relative operating efficiency of at least 40 per cent when measured according to the standardized test.
2. With forced-draft units the net-weight variation of product per square foot of tray area between trays, when loaded at the start with 1½ pounds of carrots sliced 3/16 inch thick, should not exceed 3.2 ounces at any time during the drying period.
3. The temperature on and above the trays should be controlled within a range of 4.8°C.(15°F.) and preferably 2°C.(10°F.).
4. All sections of the trays should dry uniformly.
5. With forced-draft units the motor, thermostat, and heating element should be designed to stand up under the operating conditions to which they are subjected.
6. The general construction of the dehydrator and its parts should be durable, should not present a fire hazard, and should provide for simplicity of operation, handling, and cleaning.
7. The finished product should be of good quality.

SUMMARY

A method has been described which may be used for evaluating the performance of home dehydrators. Based on this test, minimum performance requirements for a home dehydrator are suggested. Data are also presented to show the influence of different products and tray loadings on the operation of home dehydrators.

It has been found that forced-draft home dehydrators of good design are most satisfactory for dehydrating foods in the home.

Natural-draft dehydrators, if properly designed, have also been found to yield good results but with this type of unit the drying time is longer, attention must be given to the control of the temperature, and the trays must be rotated at one- or two-hour intervals. In general, oven drying is least satisfactory because of the difficulty in controlling oven temperatures, the temperature variation in different parts of most ovens, and the frequency with which the trays must be rotated and shifted.

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COBALT CONTENT OF SOME FOOD PLANTS

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The essentiality of cobalt in animal growth and health has been well established as a result of studies of troubles of a certain type in grazing animals in different parts of the world. In each instance it was recognized that these troubles, later overcome with the use of cobalt, were limited to certain areas of the country in question, and a recognized cure before the cause of the disease was known was to shift the cattle or sheep to other areas, often nearby, that were not associated with the disease.

The occurrence of cobalt in soils and plant materials has not been studied extensively. Washington (1925) estimated that there is about 0.23 per cent of cobalt in the whole earth; Goldschmidt (1937) suggests that a large part of this cobalt, owing to equilibria of partition between metallic or semimetallic phases and silicate phases, has been eliminated from the earth's crust, and consequently the cobalt content of the crust does not exceed 40 grams per ton or approximately 0.004 per cent. The early work of Robinson (1914) and of Bertrand and Mokragnatz (1922a, 1922b, 1924, 1925) indicates, however, that cobalt must be widely distributed in both soils and plants regardless of their origin or kind. Bertrand and Mokragnatz, for example, found cobalt in all of 20 samples of the edible portions of plants collected from different soils except one sample of carrots. They also observed that the parts of the plant have somewhat the following relative standing with respect to their content of cobalt: leaves > seeds > fruits > roots and tubers. In the stem of the plant, cobalt was found to be more abundant in the wood than in the bark, and the integuments of the grain contained about the same amount of cobalt as did the kernel in the samples of wheat and oats examined by them.

Although many investigators throughout the world have reported on the cobalt content of pasture herbage, few reports other than Bertrand's have been made on cobalt in human foods. Ahmad and McCollum (1939) published data on the cobalt content of beans, peas, corn, and wheat and other foods from various parts of the United States. Their data also indicate a widespread distribution of this element.

In this laboratory an investigation is under way to establish patterns of distribution in the United States of the nutritionally important micronutrient elements. Since less is known of cobalt and its occurrence in soils and plants than of some other elements, more emphasis has been placed upon its determination at this time. Furthermore, it has been possible through the co-operation of various experiment stations to obtain samples of food materials that had already been used for studies of other micronutrient elements, such as iron, copper, and zinc. The data obtained from a study of these and other samples are reported in this paper.

EXPERIMENTAL PROCEDURE

The origin of each sample is shown in the tables. The turnip greens and spinach from Mississippi were submitted for this work by Miss Olive Sheets, Department of Home Economics, Mississippi Agricultural Experiment Station. The materials from Texas were received from Dr. Jessie Whitacre, Division of Rural Home Research, Texas Agricultural Experiment Station. The turnip greens from Virginia were submitted by Prof. H. H. Hill, Virginia Agricultural Experiment Station. All of these materials were produced in connection with a co-operative effort of several southeastern stations in studies of mineral and vitamin contents of vegetables. Various samples from Coffee County, Alabama, and Giles County, Tennessee, were collected by C. A. Kinser of the National Institute of Health, in connection with certain surveys of those counties. The samples were submitted by Dr. W. H. Sebrell of the National Institute of Health. The mangel-beet leaves from Rhode Island were obtained from Vice Director B. E. Gilbert of the Rhode Island Experiment Station. They were a part of a study of the effect of one crop on the yield and quality of the succeeding crop. The samples from Massachusetts were obtained from Prof. W. S. Ritchie, Department of Chemistry, Massachusetts State College. These samples, which have been described and analyzed by Holland and Ritchie (1941), may be identified in their publication by the Massachusetts numbers given (Table 1). The spinach leaves and mangel-beet leaves from Storrs, Connecticut were submitted by Prof. B. A. Brown, Department of Agronomy, Connecticut Agricultural Experiment Station.

The turnip greens from Mississippi were washed in tap water immediately after harvesting, followed by rinsing in distilled water. The excess of water was removed by blotting them with cheesecloth, and the samples were dried and ground in an Abbé mill. The preparation of samples from other sources was essentially the same. Hood, Parks, and Hurwitz (1943) have found that the ball mill with flint balls contributes some cobalt to the sample in the course of grinding, particularly when the sample is small. A maximum contamination of 0.03 microgram of cobalt per gram of plant material was obtained in a sample of 120 grams ground for 19 hours. Other conditions resulted in a greater or less contamination, but these conditions were more typical for the materials used in this study. It is evident from the data (Table 2) that an error of this magnitude is not serious except for samples that are very low in cobalt.

Two methods of analysis were used: (a) the nitroso-R-salt method as modified by Kidson and Askew (1940) and (b) the o-nitroso cresol method as developed by Thompson and Ellis (1943) from the original nitrosophenol method of Cronheim (1942). The agreement between the methods was excellent, but the method of Thompson and Ellis has the following advantages: (1) there is greater ease of manipulation, (2) the color intensity can be determined in a photoelectric colorimeter, and (3) it is very sensitive and thus a smaller sample can be taken for analysis.

DISCUSSION OF RESULTS

An examination of the average cobalt contents along with their standard errors (Table 2) reveals a significant difference between certain groups of

TABLE 1

Cobalt Content of Some Foods

(Moisture-free basis)

Materials	Source	Description	Number of samples	Co (p.p.m.)		
				Max.	Min.	Av.
Beans, wax.....	Mass. State College	Strings removed; harvested 8/1/34; Mass. No. 62; grown in New England upland section.	1	.....	.....	0.10
Beets, roots.....	Mass. State College	Pared; harvested 7/12/34; Mass. No. 65.	1	.....	.....	0.05
Beets, roots.....	Mass. State College	Boston Crosby; scraped; harvested 8/6/35; micronutrients applied; Mass. No. 352, 353, 354, 355.	4	0.09	0.06	0.07
Beets, roots.....	Mass. State College	Ditto but no micronutrients; Mass. No. 356.	1	.....	.....	0.09
Beets, tops.....	Mass. State College	Boston Crosby; mature; crops of 1935 and 1936; micronutrients applied; Mass. No. 358, 359, 360, 488, 489, 490, 491.	7	0.55	0.25	0.41
Beets, tops.....	Mass. State College	Ditto but no micronutrients.	2	0.45	0.34	0.39
Cabbage.....	Coffee Co., Alabama	Edible portion of mature head; crop of 1939; grown in East Gulf coastal plain.	3	0.27	0.12	0.21
Cabbage.....	Giles Co., Tennessee	Edible portion of mature head; crop of 1939; grown in Nashville basin.	5	0.50	0.10	0.24
Cabbage.....	Giles Co., Tennessee	Edible portion of mature head; crop of 1939; grown in highland rim section.	1	.....	.....	0.22
Cabbage.....	Mass. State College	Crops of 1933 and 1937; Mass. No. 2 and 688; grown in New England upland section.	2	0.10	0.09	0.09
Cabbage.....	Mass. State College	Mass. No. 689, 698, 699; grown in seaboard lowland section.	3	0.18	0.06	0.13
Chard, Swiss...	Mass. State College	Mass. No. 3 and 87.	2	0.13	0.06	0.09
Corn, field.....	Coffee Co., Alabama	Mature grain; grown in East Gulf coastal plain.	9	0.03	0.01	0.02
Corn, field.....	Giles Co., Tennessee	Mature grain; grown in Nashville basin.	6	0.01	0.01	0.01
Corn, field.....	Giles Co., Tennessee	Mature grain; grown in Highland rim section.	2	0.01	0.01	0.01

TABLE 1 *(Continued)*

Cobalt Content of Some Foods

(Moisture-free basis)

Materials	Source	Description	Number of samples	Co (p.p.m.)		
				Max.	Min.	Av.
Corn, field	Giles Co., Tennessee	Mature grain; grown in alluvial soil.	2	0.01	0.01	0.01
Cowpeas	Coffee Co., Alabama	Mature seed; crop of 1937; grown in East Gulf coastal plain.	7	0.22	0.04	0.13
Cowpeas	Giles Co., Tennessee	Mature seed; crop of 1937; grown in Nashville basin.	5	0.15	0.07	0.10
Cowpeas	Giles Co., Tennessee	Mature seed; crop of 1937; grown in highland rim section.	1	.....	.....	0.06
Cowpeas	Giles Co., Tennessee	Mature seed; crop of 1937; grown on alluvial soil.	1	.....	.....	0.17
Cowpeas	College Sta., Texas	Mature seed; crops of 1939 and 1941; grown on West Gulf coastal plain on Lufkin fine sandy loam.	4	0.38	0.26	0.31
Lettuce	Mass. State College	Boston head; edible portion, good condition, 1933; Mass. No. 4; grown in New England upland section.	1	.....	.....	0.20
Lettuce	Mass. State College	New York 12; micronutrients applied; Mass. No. 322, 323, 324, 325, 468, 469, 470, 471.	8	0.31	0.13	0.21
Lettuce	Mass. State College	Ditto but no micronutrients; Mass. No. 326 and 472.	2	0.33	0.14	0.23
Mangel-beet leaves	Storrs, Conn.	Crop of 1942; grown in New England upland section on Charlton fine sandy loam.	10	0.71	0.41	0.54
Mangel-beet leaves	Kingston, R. I.	Crop of 1942; grown in seaboard lowland section on Bridgehampton very fine sandy soil.	6	0.19	0.05	0.16
Spinach	State College, Miss.	Aragon (Hastings) variety; cut ½ to ¾ inch above crown of root, yellow leaves discarded; plants just past prime for market; crop of 1941; grown in East Gulf coastal plain on Ochlockonee fine sandy loam; nitrogen applied.	4	0.84	0.55	0.70
Spinach	State College, Miss.	Ditto but no nitrogen.	1	.....	.....	1.20

TABLE 1 *(Continued)*

Cobalt Content of Some Foods

(Moisture-free basis)

Materials	Source	Description	Number of samples	Co (p.p.m.)		
				Max.	Min.	Av.
Spinach	Mass. State College	Crop of 1934; Mass. No. 90 and 91; grown in New England upland section.	2	0.29	0.28	0.28
Spinach	Mass. State College	Burpee's Victoria; micronutrients applied; Mass. No. 316, 317, 318, 319.	4	0.43	0.41	0.42
Spinach	Mass. State College	Ditto but no micronutrients.	1	.....	.....	0.40
Spinach	Storrs, Conn.	Grown in New England upland section; micronutrients applied.	6	0.63	0.50	0.55
Spinach	Storrs, Conn.	Ditto but no micronutrients.	2	0.52	0.50	0.51
Spinach, New Zealand	Mass. State College	Crops of 1933 and 1934; beyond edible stage, seed formed; Mass. No. 11 and 81; grown in New England upland section.	2	0.10	0.09	0.09
Sweet potatoes	Coffee Co., Alabama	Crop of 1937; edible portion.	7	0.05	0.02	0.03
Sweet potatoes	Giles Co., Tennessee	Crop of 1937; edible portion.	6	0.05	0.01	0.02
Turnip greens	Mississippi	Seven-top variety; leaf blade only; planted in fall of 1938; mature but still marketable; fertilizer applied; grown in East Gulf coastal plain.	3	0.15	0.08	0.10
Turnip greens	Virginia	Seven-top variety; leaf blade only; grown at Norfolk, Va.	2	0.08	0.08	0.08
Turnip greens	Virginia	Seven-top variety; leaf blade only; grown at Blacksburg, Va.	1	.....	.....	0.03
Turnip greens	Texas	Seven-top variety; leaves only; fall crops of 1938 and 1939; irrigated; grown in the Osage plains of the Central Lowland Province on Yahola loamy very fine sand.	2	0.80	0.27	0.43
Turnip greens	Texas	Seven-top variety; leaves only; fall crops of 1938 and 1939; irrigated and fertilized; grown in West Gulf coastal plain on Orelia clay loam.	3	0.25	0.10	0.20

TABLE 1 *(Concluded)*

Cobalt Content of Some Foods

(Moisture-free basis)

Materials	Source	Description	Number of samples	Co (p.p.m.)		
				Max.	Min.	Av.
Turnip greens.....	Texas	Seven-top variety; leaves only; planted in fall of 1938; large, tender leaves; grown in West Gulf coastal plain in Lufkin fine sandy loam.	2	1.25	0.90	1.07
Turnip greens.....	Texas	Four varieties all grown under similar conditions; crop of of 1941; no fertilizer applied; grown in gulf coastal plain.	4	0.32	0.24	0.28
Turnip greens.....	Texas	Ditto but fertilized.	4	0.45	0.43	0.44
Turnip greens.....	Texas	Crop of 1940; composite samples of 16 treatments each; grown in gulf coastal plain.	3	0.37	0.26	0.30
Turnip greens.....	Mass.	Above-ground portion of plant; fall crop of 1940; gypsum applied; grown in New England upland section.	4	0.40	0.24	0.30
Turnip greens.....	Mass.	Ditto but no fertilizer.	1	.....	.....	0.92
Turnip greens.....	Mass.	Ditto with phosphate applied.	2	0.30	0.27	0.28

materials used in this study. The cobalt content of the spinach is significantly higher than that of any other food examined. The group containing mangel-beet leaves, beet tops, and turnip greens contains significantly more of this element than the remaining groups, and the group including the root crops and field corn falls in the lowest category. The beet tops contain nearly six times as much cobalt as their roots, and in general, the data indicate that the leafy foods contain the highest amount of cobalt.

Numerically the data reported here are not sufficient to be useful in any detailed soil studies but, in general, they indicate that certain regional differences in cobalt content of foods may exist. Thus, where the cobalt contents of turnip greens from different natural regions are summarized (Table 3) it is clear that those samples collected from certain parts of the coastal plain contain much smaller quantities of cobalt than do those from other areas. This regional difference is further emphasized in the cobalt content of mangel-beet leaves (Table 1) where it is apparent that those produced in the New England upland contain more cobalt than do those grown in the Atlantic coastal plain. It should be noted, however, that the samples of spinach (Table 1) from Mississippi contain as much or more cobalt as do those from Massachusetts and Connecticut.

There is some indication in these data that a deficiency in the soil of a macronutrient element, such as nitrogen that limits the growth of the plant, may result in a higher cobalt content of the unfertilized plant, particularly where the supply of cobalt is limited. Thus, the unfertilized spinach from State College, Mississippi (Table 1) contains 1.20 p.p.m. of cobalt, while the application of nitrogen resulted in an increased yield

TABLE 2

Summary of Cobalt Content of Samples Investigated According to Species

(Moisture-free basis)

Species	Number of samples	Cobalt
		p.p.m.
Group I		
Spinach	20	0.67 ± 0.05[1]
Group II		
Mangel-beet leaves	16	0.40 ± 0.05
Beet tops	9	0.40 ± 0.03
Turnip greens	31	0.34 ± 0.04
Group III		
Lettuce	11	0.21 ± 0.07
Cabbage	14	0.19 ± 0.03
Cowpeas	18	0.16 ± 0.02
Group IV		
Beets, roots	6	0.07 ± 0.01
Sweet potatoes	13	0.03 ± 0.01
Corn, field	19	0.01 ± 0.002

[1] Mean with standard error.

TABLE 3

Cobalt Content of Turnip Greens, Summarized According to Natural Regions

Region	Number of samples	Cobalt
		p.p.m.
East Gulf coastal plain	3	0.10
Atlantic coastal plain	2	0.08
Central lowland	2	0.43
West Gulf coastal plain	16	0.41
New England upland	7	0.38

but a much lower content of cobalt. Many of the materials from Massachusetts State College were grown in lysimeter plots with applications of iron, copper, manganese, or iodine [Holland and Ritchie (1941)] but neither the summaries in Table 1 nor the more detailed unpublished data show any effect of these micronutrients on the cobalt content. The spinach from Connecticut had also received applications of boron and manganese, but no relationship to cobalt content is apparent. None of these materials was grown on soils fertilized with cobalt compounds. It was found by one of us (Beeson), however, that lettuce grown on the Chester loam in Virginia contained 0.3 p.p.m. of Co (dry basis) with no cobalt applied to the soil, and 5.1 p.p.m. of Co when four grams of cobalt sulfate was added to an area of soil containing 20 heads of lettuce. This was an excessive

application, but it indicates that cobalt is readily absorbed by the plant when a quantity of it is present in the soil. This is in agreement with many pasture investigations particularly in New Zealand.

The effect of the stage of maturity, the cultural practices, and climatic conditions cannot be evaluated from these data since they were not observed where location, soil type, and other factors were maintained as constants.

In the evaluation of data of the kind presented here, particularly with respect to regional differences, it is of utmost importance to observe and note all of the available information concerning those factors likely to modify the composition of the plant. In this laboratory, the following information is considered to be essential in interpreting analytical data on plant material:

1. COMMON AND SCIENTIFIC NAMES: The type or variety and strain should be noted if possible.

2. DESCRIPTION OF SAMPLE:
   (a) *Stage of maturity.* This should be related as definitely as possible to the appearance or development of some organ of the plant as the bud or the seed with descriptions of the stage of maturity as "in milk stage." Terms such as "mature as for hay" or "marketable as for food" are not very definite unless management practices and market requirements are very well standardized.
   (b) *The part of the plant taken* should be carefully described, and wherever possible, it should be a definite organ. If a portion only of the leaves, for example, is selected, their position on the stem should be recorded.
   (c) *The number of cuttings of forages and the yields* might be noted when the information is available.
   (d) *The condition of the plant at harvest.* Observations as to a diseased condition or insect injury should be made. The relative size of the plant, the proportion of leaves to stem, and the general marketable qualities might be noted.
   (e) *Treatment after harvest.* The methods of removing soil contamination, drying, and grinding should be recorded.

3. SOURCE OF SAMPLE: It is very important to describe as accurately as possible the location of the plot or field from which a sample was obtained. Where practicable, the locations should be indicated on detailed soil maps of a scale of at least one inch to the mile. A careful description also makes it possible to obtain additional samples from the same location.

4. DESCRIPTION OF THE SOIL: The name of the soil type as classified by the U. S. Department of Agriculture, Soil Survey, is preferred. Such terms as loam, sand, or clay have much less significance although where no soil survey has been made, they may be useful if an accurate description of the location and other soil characteristics is made.

5. CULTURAL INFORMATION:

(a) *Fertilizers applied.* Particular attention should be given to the use of the micronutrient elements. The use of sprays should be noted.

(b) *History of the plot or field.* This should include the crop rotations used and past fertilizer applications.

(c) *Rainfall or other climatic data.* The distribution as well as the quantity of rainfall is of importance in certain regions. The use of irrigation waters should be noted.

(d) *The dates of planting and harvesting* are sometimes useful information.

It is recognized, of course, that it is not possible always to collect all of this information, but the recording of a substantial part of it will contribute greatly to the value of the analytical data.

The information accompanying the materials used in this study was unusually complete with respect to descriptions of the sample, its condition at harvest, and the location and soil type where it was grown. Additional information would aid in interpreting, for example, the differences found that seem to be related to fertilizer applications. Thus, differences in distribution of rainfall throughout the growing season might so affect composition as to entirely obliterate any soil effect as between two localities. Likewise, differences in the stage of maturity or the time of harvest might easily account for important variations in the mineral content of the plant. It is recognized, of course, that in the final analysis the true evaluation of the relationship of soils or any factor to the nutritive value of plants must rest upon two fundamental steps: first, that the plant must be grown under carefully controlled conditions with this objective in mind and second, the nutritive value must be biologically measured. Thus, the response of an animal to differences in the cobalt content of a plant could be the result of many factors of which the total amount present is only one.

SUMMARY

The cobalt contents of a number of common food plants have been presented. These materials were carefully selected from various regions of the United States and give some indication of environmental factors which may influence the cobalt content of a plant. The information essential to a proper evaluation of data of this kind is discussed.

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A STUDY OF PRESERVATION OF EGGS BY FLASH HEAT TREATMENT

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The main objectives in preservation of eggs for human consumption are as follows: (1) to slow down the rate of physical and chemical deterioration, (2) to preserve the nutritive value and cooking characteristics, and (3) to retain the natural appearance of egg contents. To achieve these objectives the eggs must be guarded from possible physicochemical changes resulting from contact with dry, liquid, or gaseous preservatives as well as from adulteration during actual processing. Cold storage, although the most natural, is effective only for a relatively short time; therefore a simple, practical method for extending the use of cold storage, or for keeping at higher temperatures for a limited time when cold storage is not available, is much desired.

Among numerous methods, heat treatment of eggs is of more recent interest and promise. In fact, there have been many attempts made to use heat in preservation of eggs. Benjamin (1914) stated that "if an egg is dropped into water of just the right temperature, a very thin film of hardened albumen is formed just inside the shell, and this makes the egg yolk appear relatively less distinct and the egg consequently fresher than it really is." Cadet de Vaux [quoted by Leach (1920), p. 1090] suggested that a heat-coagulated thin layer of white next to the shell forms an impervious coating inside the shell.

Recently Funk (1943a) made a study of preservation by submerging eggs into oil at 60°C.(140°F.) for about 10 minutes to cause a slight coagulation of the entire egg albumen. It is possible that partial reversion in this coagulated albumen may occur with aging as it does in other proteins—albumins [Adolph (1926)]. It was observed that the treated fertile eggs could be kept in "good edible condition" for six weeks at as high a temperature as 29.4°C.(85°F.). He emphasized [Funk (1943b)] that there is a complete arrest of embryonic development and check in bacterial growth. The former is undoubtedly an essential factor in preserving fertile eggs during hot summer months. As far as the bacterial growth is concerned it has been shown by Wedeman (1940) that eggs inoculated with *paratyphus-enteritis* group before "pasteurization" by a somewhat more drastic method—heating for 20 minutes at 65°C.(149°F.)—were not made sterile.

Considering the general importance and applicability of this method for commercial use Barott and McNally (1943), using Funk's conditions of treatment, performed numerous tests on temperature coagulation (opacity) of egg albumen. From this study they concluded that there are several variables that may interfere with securing uniform results by this slow heating method. The more important of these are initial temperature, size, and freshness of the egg. To overcome these variables would

require more accurate control of temperature according to the condition of eggs at the time of their treatment.

Along a somewhat similar line of endeavor we have been carrying on experimental work at Cornell. Our chief objectives, however, were (1) to determine temperature and time of exposure required to coagulate a very thin layer of outer albumen, which may serve as a protective film to the contents of the egg, and (2) to evaluate the effectiveness of such treatment in preservation of eggs held for human consumption.

MATERIAL AND METHODS

Over 950 White Leghorn (*Gallus domesticus*) eggs, which immediately after laying were held at room temperature for at least two hours, were treated in small lots (about 18 eggs) at temperatures from 60°C.(140°F.) to 100°C.(212°F.) for various periods, up to one minute. In treatment each lot of eggs was placed in a single layer in a wire basket and immersed in a thermostatically controlled water bath. They then were air cooled and, together with control lots, stored at room temperature, 21°C.(70°F.), and at refrigerator temperature, 5°C. (41°F.), for as long as 12 months.

In the examination of treated and control lots of eggs observations were made for the appearance of coagulated albumen, physical condition, color and opacity of albumen, color and shape of yolk, and odor of egg contents.

The beating power of preserved eggs was measured by beating the whole egg white for five minutes in a calibrated measuring cylinder, at the motor speed of about 1,200 r.p.m. (Fig. 1). The volume was read directly and relative increase was calculated using the following formula: $\frac{V_f}{V_a} \cdot 100$, where V_f is volume of the foam, and V_a is volume of the sample of albumen.

EXPERIMENTAL RESULTS

Effect of One-Minute Treatment at Various Temperatures: To determine the general effect of heat treatment on preservation, eggs were immersed in water at temperatures of from 60°C.—just below the coagulation point [Romanoff (1943a)]—to 100°C. for one-minute periods. Then the eggs were stored at room temperature of 21°C. and at the cold-storage temperature of 5°C. At the end of one year all eggs, treated and untreated, were examined.

From the summary of data presented (Table 1) it will be seen that the quality of treated and stored eggs deteriorates less with the increase in treating temperature. After treatment at 100°C. they were well preserved during the 12 months of storage at 5°C. and were in relatively good condition even when stored for the same period at room temperature of 21°C.; however, the harmful effects of high storage temperature on general quality of eggs was evident.

In refrigerated eggs, treated at 100°C., the appearance of the yolk was good, showing only slight change in color and retaining good shape. The consistency of the albumen was somewhat thinner and the odor was hardly noticeable. All these conditions point out the efficiency of high-

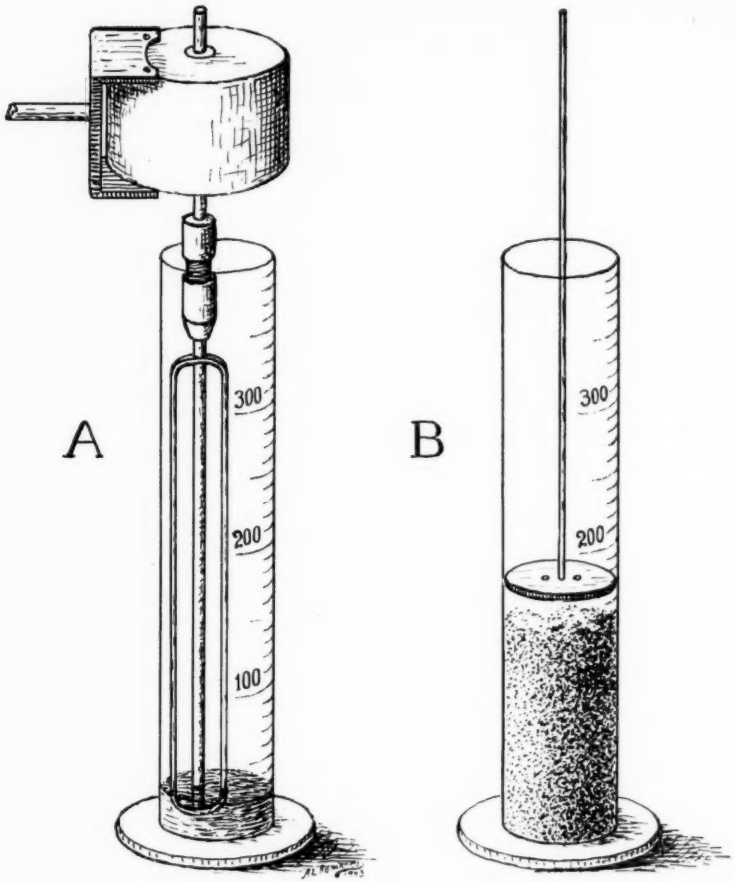

FIG. 1. A laboratory method for the quantitative measurement of beating quality of eggs: A, the arrangement of an electric motor with a narrow-bladed beater placed into a shortened 500-c.c. calibrated measuring cylinder; B, the compression of scattered material with a light plunger, having holes near center of disc to allow the escape of air, for the direct reading of the volume of beaten egg.

The procedure is as follows: an accurately measured sample of material is transferred into the cylinder; the beater is lowered in an accentric position to insure a more effective beating; after the material has been beaten the beater is removed while still in motion—in this manner only a negligible amount of material is left adhering to the blades; then a light circular plunger (diameter close to that of the cylinder) is used to compress the foam clinging to the sides of the vessel; and from the level of the plunger a direct reading of the volume is made.

This direct reading of the volume of foam makes for a more rapid and more accurate measure than the transfer and weighing of an aliquot, as in the methods previously described by St. John and Flor (1931), Henry and Barbour (1933), and Bailey (1935).

temperature treatment on the preservation of eggs. However, one objection was observed in some of the opened eggs; that is, the presence of a layer of coagulated albumen.

From further tests it was found that fresh eggs immersed for one minute in water at 60°C. and opened immediately showed no film of coagulated albumen. At 70°C. there was a slightly visible film of coagulated albumen, and at 80°C. the film was thicker and more distinct. The effect of coagulation was still more evident at 90°C., and at 100°C. a thick layer of coagulated outer albumen closely adhered to the shell membrane. Treated eggs at all temperatures, held for one week, showed practically the same results as the fresh eggs.

TABLE 1

Results of One Minute of Heat Treatment at Various Temperatures on Preservation of Eggs Held 12 Months in Storage

Treated one minute at	Stored at	Condition of 12-months-old eggs			
		Yolk		Consistency of albumen	Odor of eggs
		Color	Shape		
60°C.(140°F.)	21°C. (70°F.)	Very deep	Much flattened	Watery	Very strong
70°C.(158°F.)		Deep	Flattened	Watery	Decided
80°C.(176°F.)		Medium deep	Some flattened	Watery	Distinct
90°C.(194°F.)		Medium	Slightly flattened	Slightly watery	Faint
100°C.(212°F.)		Medium light	Fairly good	Very slightly watery	Very faint
60°C.(140°F.)	5°C. (41°F.)	Medium	Some flattened	Little watery	Faint
70°C.(158°F.)		Medium	Slightly flattened	Fairly good	Very faint
80°C.(176°F.)		Medium light	Fairly good	Fairly good	Very faint
90°C.(194°F.)		Medium light	Good	Good	Normal
100°C.(212°F.)		Light	Good	Good	Normal

Lowering the Visible Layer of Coagulated Albumen: Since preserving power was found to be best at 100°C.(212°F.) it was decided to determine how much the time of exposure at this temperature could be shortened thus to minimize the appearance of visible layer of coagulated albumen in an opened egg. While this thin layer presumably does not reduce appreciably the usefulness of the egg as an article of food, nevertheless such eggs may be discriminated against by persons not familiar with the underlying principles of this method of preservation.

The results of a search for the longest exposure at which there would not be any visible coagulated albumen are summarized (Table 2). The observations show that eggs, opened immediately after treatment for two seconds, already have slight signs of coagulation; but if the treated eggs are opened after seven days of storage, either at room or refrigerator temperatures, the flakes of coagulated albumen occur only in eggs exposed for periods over five seconds. It was also evident that the film of coagulated albumen, with cooling and aging, adheres to the shell membrane. This adherent film of coagulated albumen can, however, be easily washed away with a stream of water from the interior surface of the broken shell. Further tests also showed that the adherence of the film of coagulated

albumen to the inner shell membrane occurs within one or two days after the treatment.

TABLE 2

Influence of Period of Treatment of Fresh Eggs at 100°C.(212°F.) on Appearance of Coagulated Albumen

Period of treatment at 100°C.	Amount of visible coagulation[1]	
	Opened immediately	Opened after one week
sec.		
1	—	—
2	+	—W
3	++	—W
4	+++	—W
5	++++	—W
7	++++	+
9	+++++	+
11	+++++	++
15	+++++	++
20	+++++	+++

[1]— invisible; + slightly visible; ++ more visible; +++ cloudy layer; ++++ white layer; +++++ distinct white layer; W means the coagulated film of albumen adheres to the shell and can be washed away from it in flakes.

On the whole, these studies indicate that a five-second exposure at 100°C.(212°F.) is the upper limit in heat treatment of eggs by this method, in order to avoid the undesirable appearance of a film of coagulated albumen.

Effect of a Five-Second Treatment at 100°C.: Now it is of interest to determine the effectiveness of a treatment of eggs for five seconds in boiling water (at 100°C.) on their preservation. With this idea in mind, fresh treated eggs were stored together with control lots in a refrigerator at 5°C.(41°F.) and at room temperature of 21°C.(70°F.). At the end of 12 months of storage all eggs were examined; the results of such a study are summarized briefly (Table 3).

TABLE 3

Results of Five Seconds of Heat Treatment at 100°C.(212°F.) on Preservation of Eggs During 12 Months of Storage

Type	Stored at	Condition of 12-months-old eggs				
		Diameter of air cell	Yolk		Albumen	Odor
			Color	Shape		
		cm.				
Treated....	21°C.(70°F.)	3.93	Medium light	Flat	Clear, watery	Decided
Control.....		4.13	Very dark	Broken	Pink, watery	Very strong
Treated....	5°C.(41°F.)	3.20	Light	Stand well	Well preserved	Normal
Control.....		3.25	Dark	Flattened	Clear, watery	Faint

Treated eggs, stored in refrigerator, upon examination showed well-preserved albumen, light and well-shaped yolk, and gave no objectionable odor. Untreated eggs, under similar conditions of storage, showed watery albumen and distinctly dark color of yolk. In addition, the yolk was

frequently stuck to the shell and upon opening the vitelline membrane had ruptured. The odor was very pronounced.

All indications are that five seconds' treatment is efficient in preserving the eggs. It was estimated that the quality of 12-months-old treated eggs is equivalent to about that of three-months-old untreated eggs kept under similar refrigerating conditions.

It is of interest to note that even under the more extreme conditions of storage, at room temperature of 21°C.(70°F.) over a 12-months period, the treated eggs showed less deterioration than untreated eggs. In quality the treated eggs were approximately equal to untreated ones held for six months.

Beating Properties of Treated Eggs: Since beating or foaming properties is one of the usual criteria of storage eggs, tests were made to determine the effect of heat treatment on beating power of egg white.

TABLE 4

Beating Properties of Hen's Egg Albumen[1]

Series of tests	Increase in volume with beating					
	Fresh			Stored for two weeks at 21°C.		
	Untreated[2]	Treated for 5 seconds at 100°C. (212°F.)	Treated for 10 minutes at 60°C. (140°F.)	Untreated[2]	Treated for 5 seconds at 100°C.	Treated for 10 minutes at 60°C.
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
1	340	360	200	400	460	400
2	312	420	240	420	420	380
3	383	400	300	480	500	240
4	350	.....	.....	400	460	320
5	390	.....	.....	380	400	320
Averages	355	393	247	424	448	340

[1] 25 c.c. of albumen (composite samples of two to nine eggs) beaten at 1,200 r.p.m. for five minutes. [2] Composite samples of all layers of albumen.

Preliminary tests have shown that there is a slight variation in beating properties of different layers of albumen. Indications are that the highest value in percentage of increase in volume with beating is in the outermost thin layer (372 per cent), then in the middle thick (359 per cent) and middle thin (320 per cent) layers, and finally the lowest value is found in the innermost chalaziferous layer. The layers have been separated by the pipette method [Romanoff (1943b)].

Samples in which the albumen has been separated by the screen method into two fractions—thick and thin (a combination of outer thin and middle thin layers)—showed a tendency towards higher beating properties of the thin fraction [St. John and Flor (1931) and Bailey (1935)].

Since the differences in foaming power of the first three layers of the egg white are not great, the present study was made on composite samples of the entire albumen, excluding, however, the inner chalaziferous layer and the chalazas.

The beating properties of the albumen of fresh and stored eggs under flash (five seconds at 100°C.) and holding (10 minutes at 60°C.) methods

are shown (Table 4). From the eggs opened immediately after treatment it was clear that the flash method of treatment causes very little change in beating power. Readings in eggs held for two weeks at room temperature of 21°C.(70°F.) show a proportional increase in volume on beating similar to that of untreated eggs. The lower values in beating properties of albumen from eggs treated by the holding method may be attributed to a partial coagulation of the albumen during processing.

Effect of Heat Treatment Upon Life of Fertile Eggs: In view of the interesting results from the five-second treatment at 100°C. it seemed worth while to investigate the degree of natural denaturation, if any, resulting from such treatment. The biological test, that is, the development of the embryo, would be the most reliable measure from the nutritional point of view. Therefore, fertile eggs, after treatment at 100°C. for various lengths of time, were incubated and the progress of embryonic development recorded.

It was found that eggs treated for not over five seconds at 100°C. were able to develop and reach the stage of hatching, although the hatchability was gradually lost with the increase in the length of treatment, as shown below.

Length of exposure (sec.)	Early death (pct.)	Late death (pct.)	Hatching (pct.)
0	3.5	9.0	87.5
3	6.2	27.1	66.7
5	22.3	44.3	33.4
7	84.4	15.6	0.0
9	100.0	.....	.....
15	100.0	.....	.....
25	100.0	.....	.....

All treatments for over five seconds resulted in embryonic mortality. The exposures for nine seconds and longer have checked the development in early stages, within the first two days. Since some development has been noticed even with the longest exposure of 25 seconds, it is possible to assume that the actual treatment was not fatal to all embryos, and that the development was stopped not necessarily by the denaturation of albumen and by changes in natural biological qualities of yolk, but by the restriction in gaseous exchange of the embryo over two days of age by the film of coagulated albumen. This fact is illustrated by a slight decrease in loss of weight of treated eggs, shown by the diameter of air cell (Table 3).

In actual practice, however, the storage temperature seldom exceeds 26.7°C.(80°F.). At this temperature the development of the blastoderm in treated eggs would be hardly noticeable.

DISCUSSION

It is obvious that these studies suggest a new practical method of preservation of shell eggs by heat treatment. In this method only a very thin section, next to the shell membrane, of the outer layer of albumen is coagulated, forming a protective coating without the heat denaturation of the entire egg contents (Table 3). There is but little damage in natural

consistency since many treated fertile eggs are able to hatch. This implies that the nutritive value, flavor, and cooking characteristics of the egg possibly remain least altered.

In the practice of preservation the use of high temperature-short period (or flash method) treatment (five seconds at 100°C.) considerably reduces many variables involved in the low temperature-long period (or holding method) treatment (about 10 minutes at 60°C.), and thus this treatment should give more uniform results. The elevation from storage or even from room temperature to the temperature of boiling-water bath is so great that the ordinary variation in the initial temperature of the egg would be of little importance. Also, since the treatment affects only the outer layers of the egg, neither the size of the egg nor thickness [Romanoff (1929)] and permeability [Romanoff (1943a)] of the shell would greatly influence the results.

The important factor to consider here is the age of the egg. It is well known that the pH of egg albumen increases with age [Sharp and Powell (1931) and Romanoff (1940)], and according to Sorensen and Sorensen (1925) the higher the pH the less heat required for coagulation. From this it is evident that older eggs would require a shorter period of exposure. However, the effect of the age of the egg again will not be so great in this method as in the case of the low-temperature treatment, as has been demonstrated by Barott and McNally (1943).

It is interesting to note that in this method the use of water at boiling temperature requires neither a thermostatic control nor stirring device. This simplifies the procedure and makes the method adaptable both for home and commercial preservation. Especially, in home preservation it is important that no elaborate equipment either for heat treatment or cooling be required; this method is easy, clean, and appeals to the aesthetic sense of every housewife. From the commercial standpoint, owing to very short period of exposure, this method should prove very efficient in operation. Exception would be in those locations which are elevated much above sea level, where boiling point of water would be below 100°C. Under such conditions it would be necessary to lengthen the period of exposure.

Furthermore, it seems that the eggs treated by this method could be kept for a reasonable length of time at ordinary room temperature. At lower storage temperatures—down to 0°C.(32°F.)—these treated eggs show superior keeping qualities over a long period of time.

The preservation of fertile eggs, intended to be kept at temperatures higher than physiological zero, that is, over 20°C.(68°F.) [Edwards (1901-02)] would require more drastic treatment, that is, longer exposures. It is evident that effective results with these eggs can be achieved only at the expense of the appearance of a thicker layer of coagulated albumen.

On the whole, the results of this method of preservation are so interesting and apparently significant that a more thorough study of the chemistry of the underlying principles would be desirable.

SUMMARY

It was found that flash heat treatment of eggs at temperatures above the coagulation point of albumen proved to be an efficient method for preservation of table eggs.

A five-second exposure of fresh eggs to boiling water forms a thin protective film of coagulated albumen, adherent to the shell membrane.

The treatment did not show any noticeable denaturation in egg contents since beating power of albumen and natural biological qualities of yolk remained unchanged.

The quality of treated eggs held in refrigerator (40°F.) and at room temperature (70°F.) was superior to untreated eggs held under similar conditions.

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A STUDY OF THE GERMICIDAL POWER OF SODIUM CHLOROORTHOPHENYLPHENATE COMPARED WITH CALCIUM HYPOCHLORITE IN THE PRESENCE OF TOMATO JUICE[1]

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The food industry has two types of safe and dependable methods of the sterilization of equipment—steam heat and some form of chlorine compound. Each has its advantages and disadvantages. Steam is usually not injurious to metal or wood equipment and when properly used is effective. When applied as a booster with hot water or detergent a good cleansing action is obtained, although the temperature on the equipment may not rise above 93.3°C.(200°F.), if it gets that high. Of course, when equipment can be readily dismantled and placed in a retort or autoclave and pressures of 10 or 15 pounds of steam applied, complete sterility can be obtained, but this is applicable to only a limited portion of the equipment used in a food plant. In closed pipe lines steam can be introduced and sufficient pressure obtained to produce sterility, but care must be used to avoid air pockets in sidelines, etc., where without steam little sterilizing action is obtained. Also with steam there is no problem of the introduction of foreign flavors.

Various chlorine compounds have been widely used in the food industry, and when all food particles have been removed previously, it is highly effective. The removal of practically all of the organic matter is important, and usually, because some particles of food remain, an extra amount of compound is added to compensate for that absorbed by the organic matter. Under operating conditions in a food plant, particularly during peak loads, the "clean up" is apt to be slighted, so that the work left to the disinfectant is much greater than was intended. Also, chlorine disinfectants cannot be used indiscriminately upon all types of equipment; advice as to the proper applications for each compound should be obtained from its manufacturer.

No good method for the disinfection of wooden sorting and trimming tables, chopping blocks, etc., has been found. Both steam and chlorine compounds have been and are used, but the cleansing action is only superficial. The wood remains moist for many hours after packing and cleaning operations are finished, and plant or animal juices will have penetrated deeper than the disinfectant.

It has recently been suggested that some of the newer germicides, whose effectiveness is not destroyed by organic matter, might be useful

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in the food industry. In a previous study [2] the authors compared the germicidal efficiencies of sodium orthophenylphenate and sodium chloro-orthophenylphenate [3] to calcium hypochlorite and sodium p-toluenesulphonchloramide in the presence of oyster liquor in an attempt to show their useful application as disinfectants in the oyster indusry. To consider the usefulness of some of these disinfectants in the food and canning industry, organic matter of vegetable origin was considered in this study.

EXPERIMENTAL PROCEDURE

The disinfectants selected were sodium chloroorthophenylphenate and calcium hypochlorite. These two disinfectants were chosen because each proved to be the superior germicide in its respective group.

The tests were carried out at the same disinfectant concentrations and temperature as the first study, but with tomato juice as organic matter and at whatever pH values were produced by the added juice. The concentrations of disinfectant, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.0063, 0.004, and 0.002 per cent, were prepared from freshly made stock solutions of the respective disinfectant.

The juice was extracted by filtering unsalted canned tomatoes through clean cheesecloth of double thickness and the solids were determined by heating duplicate weighed 10-c.c. portions of the sample in a vacuum oven at a temperature of 70°C.(158°F.) for 12 hours.

It was necessary to add a suspension of organisms to these tests since the organic matter employed does not normally contain a significantly high natural flora. *Aerobacter aerogenes* was selected as the test organism. It was grown in broth for 18 hours; then the culture was centrifugalized and the organisms resuspended in sterile distilled water. To prevent any significant fluctuation in numbers during the experiment the suspension was held in an ice bath.

All experiments were carried out in a water bath maintained at an average temperature of 20°C.(68°F.). Each test in this series contained 50 c.c. of tomato juice, a definite quantity of the desired disinfectant stock solution, and enough distilled water so that when one c.c. of the suspended organisms was added the volume in the flask was 100 c.c. Mechanical stirring was employed for about 10 seconds after the bacterial suspension was added and again just prior to withdrawing each sample. After each sample was withdrawn, dilutions were prepared, plated with Standard Methods nutrient agar, and incubated at 37°C.(98.6°F.) for 48 hours; pH tests were determined at the end of the 12-minute period, using a Beckman glass electrode, and recorded as the pH of the experiment.

DISCUSSION

The results show that sodium chloroorthophenylphenate was definitely superior to calcium hypochlorite (Table 1), even though with the former both the bacterial and the organic loads were greater than with the hypo-

[2] Nolte, W. A., and James, L. H., 1941. A study of the germicidal power of some orthophenylphenol compounds compared with standard chlorine disinfectants in the presence of oyster liquor. Food Research 6, 537-546.

[3] We are indebted to the Dow Chemical Company for these chemicals.

chlorite. A concentration of this orthophenylphenate as low as 0.0125 per cent was found to be 2.5 times more effective in a 12-minute exposure than in a three-minute exposure, whereas higher concentrations of from 0.2 to 0.025 per cent were equally effective in both three- and 12-minute exposures. This is predicated on the achievement of a 99-per cent reduction in numbers of bacteria.

TABLE 1

Comparison of Germicidal Power of Sodium Chloroorthophenylphenate and Calcium Hypochlorite at Various Concentrations Held at 20°C.(68°F.) ± 2°C.

Concentration of disinfectant	Sodium chloroorthophenylphenate—2,050,000 bacteria per c.c.[1] 2.77% total solids[2]			Calcium hypochlorite—840,000 bacteria per c.c.[1] 2.42% total solids[2]			Bacterial reduction after 12 min.
	Final pH of concentration	Bacterial reduction after 3 min.	Bacterial reduction after 12 min.	Available Cl. calculated[3]	Final pH of concentration	Bacterial reduction after 3 min.	
pct.		*pct.*	*pct.*	*p.p.m.*		*pct.*	*pct.*
.2	4.58	99	99	253.8	6.07	None	None
.1	4.56	99	99	126.9	5.00	None	None
.05	4.45	99	99	63.8	4.73	None	None
.025	4.45	99	99	31.9	4.60	None	None
.0125	4.40	38	96	16.3	4.50	5	18
.0063	4.40	....	7	8.5	4.40	24	17
.004	4.38	None	None	5.0	4.34	5	None
.002	4.35	None	None	2.5	4.30	5	None

[1] Bacterial count of diluted tomato juice-disinfectant mixture. [2] Per cent total solids in 1:2 dilution of tomato juice. [3] Concentration of available calcium for respective concentrations of disinfectant as calculated in earlier experiments (1).

Under the same test conditions, calcium hypochlorite was ineffective in bringing about any significant reduction even in the highest concentration employed (253.8 p.p.m. of available chlorine). Therefore a control experiment was carried out to note the germicidal activity of this disinfectant on a suspension of the test organism in the absence of tomato juice. The results (Table 2) give evidence that the chlorine compound was germicidal under these conditions. In concentrations as low as 0.004 per cent (five p.p.m. of available chlorine) it was effective in bringing about a 68-per

TABLE 2

Germicidal Power of Calcium Hypochlorite[1] at Various Concentrations Held at 20°C.(68°F.) in the Absence of Organic Matter

Concentration of disinfectant	Available Cl. calculated[2]	Final pH of concentrations	Bacterial reduction in 12 min.
pct.	*p.p.m.*		*pct.*
.2	253.8	10.96	99
.1	126.9	10.68	99
.05	63.8	10.33	99
.025	31.9	9.95	99
.0125	16.3	9.51	98
.0063	8.5	8.50	91
.004	5.0	7.71	68
.002	2.5	6.95	2

[1] Bacterial count of suspension-disinfectant mixture, 840,000 bacteria per c.c. [2] Concentration of available calcium for respective concentrations of disinfectant as calculated in earlier experiments (1).

cent reduction in bacterial numbers. The failure of calcium hypochlorite to show any germicidal effect in the presence of tomato juice probably was due to the failure of the chlorine concentrations used to more than satisfy the chlorine demand of the tomato-juice organic matter.

An ability to kill bacteria is important but not the sole requisite of a satisfactory disinfectant for use in a food plant. Our studies have included only two sources of organic matter: oyster liquor, which consists largely of protein, and tomato juice, which contains comparatively little protein but is high in carbohydrate content. Sodium chloroorthophenylphenate was highly effective under both conditions. It is interesting to note that the pH differed widely in the two investigations. Using oyster liquor the pH was adjusted to 6, 7, 8, and effective germicidal action was obtained in each. In the tests reported above equally effective germicidal action was obtained with the same compound at pH values that ranged near 4.4. Therefore, from these results, we may expect that effectiveness of this compound would not be destroyed by acidity or alkalinity when used under conditions that fall between pH 4.4 and 8. The instability of hypochlorite in the acid range is well known.

It is also interesting to note that considerable variation in total-solids content (mostly organic in nature) did not alter the germicidal effectiveness significantly. In the first study using oyster liquor from 0.30 to 0.40 per cent total solids was present, whereas in the study reported above approximately 2.5 per cent total solids was present. This suggests the possibility that the former disinfectant, sodium chloroorthophenylphenate, may be effective even under conditions in which inadequate removal of organic debris from foods has been practiced. This should have further study.

SUMMARY

In the presence of tomato juice diluted with an equal quantity of water, sodium chloroorthophenylphenate was superior to calcium hypochlorite. Calcium hypochlorite showed no significant germicidal activity under this condition. However, in the absence of the acid tomato juice this chlorine compound demonstrated a marked germicidal effect in concentrations of 0.2, 0.1, 0.05, 0.025, 0.0125, 0.0063, and 0.004 per cent, respectively. The pH was mostly alkaline.

Sodium chloroorthophenylphenate exerted a rapid germicidal effect since high concentrations were just as effective in a three-minute exposure period as in a 12-minute exposure period.

IMPORTANCE OF BREEDING PEAS AND CORN FOR NUTRITIONAL QUALITY

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It has been common knowledge for some time that the vitamin content of vegetables varies with variety as well as with other factors, such as maturity; however, the magnitude of this variation may not be appreciated in many instances.

The present study has been made to determine variations that might exist in the vitamin content of a number of breeding stocks and trial varieties of peas and corn suitable for canning. These data would be evaluated along with other desirable characteristics in selecting strains suitable for canning or breeding such strains. The purpose of this paper is to point out the importance of breeding for nutritional quality as well as for other more obvious factors.

EXPERIMENTAL PROCEDURE

The breeding lines represented here were raised under identical cultivation conditions on a light sandy loam with adequate fertilization. Three harvests were made from each lot, and the sample for analysis was taken from the harvest closest to canning maturity.

Since many of the samples were small, it was necessary to conserve material. All samples were first ground for five minutes in a Waring blender with an equal weight of an oxygen-free, three-per cent oxalic acid solution in an inert atmosphere. From this starting material samples were weighed directly into the extracting vessels for carotene, thiamin, and ascorbic acid determination. Another part of the sample was autoclaved for 10 minutes at 15 pounds pressure with an equal weight of buffer of pH 4.5 and then ground for determination of riboflavin and nicotinic acid.

Carotene was determined by saponification of the sample with 10 per cent potassium hydroxide in anhydrous methanol, followed by extraction with Skellysolve B. The carotene solution was purified by the method of Moore (1940) and the carotene determined on a Coleman spectrophotometer against a standard curve. In the case of corn samples the purification was by the methanol washing method.

Thiamin was determined by the chemical method of Conner and Straub (1941). Ascorbic acid was determined by the direct indophenol titration, using a three-per cent solution of metaphosphoric acid extraction.

Riboflavin and nicotinic acid were determined microbiologically by a modification of the method of Snell and Strong. Determinations on all samples were started within two hours of the time samples were collected in the field. This insured a minimum loss of vitamin.

RESULTS

Carotene in peas varies over 300 per cent from the lowest to the highest (Table 1). Part of this variation may be due to the difference of maturity, but this factor cannot account for more than a small portion of the difference, since all samples were taken at approximately canning maturity. In Table 2 the differences are even greater; Lot I (Table 1) represents three maturities, I (a) being very young, I (b) young, and I (c) nearly mature. It is evident here that very young peas are relatively low in carotene content. (The results in all tables are stated in micrograms per gram of fresh weight.)

TABLE 1

Vitamin Content of Peas

Culture No.	Carotene	Vitamin C	Thiamin	Niacin	Riboflavin
	γ/gm.	γ/gm.	γ/gm.	γ/gm.	γ/gm.
5A	1.39	166	2.74	30.5	1.86
14A	2.49	121	3.45	34.0	1.90
15A	2.95	212	2.26	32.5	1.72
16A	4.22	286	2.44	27.7	2.20
34B	3.80	337	3.10	29.2	1.88
66A	4.02	268	2.61	29.2	2.10
91A	4.08	322	1.79	32.9	2.30
92A	2.39	264	.....	28.5	2.43
93A	3.71	286	1.82	34.2	1.81
94A	2.77	303	.....	35.1	2.53
Lot A	4.17	262	1.37	30.2	2.57
Lot B	3.73	261	1.46	32.3	2.10
Lot C	3.57	261	1.96	28.5	2.12
Lot D	3.71	247	2.39	32.0	1.90
Lot E	3.71	248	2.44	31.1	2.01
Lot F	2.31	314	3.35	33.0	2.23
Lot G	3.64	264	.....	.....	.....
Lot H	3.36	243	.....	.....	.....
Lot I (a)[1]	1.75	261	.....	.....	.....
Lot I (b)	2.79	300	4.29	31.0	2.17
Lot I (c)	3.51	282	3.58	34.3	2.27

[1] a = very young; b = young; c = nearly mature.

The results on ascorbic acid are self-explanatory, as are those on thiamin. The values are quite variable for these, giving further emphasis to the differences in nutritional value between varieties. The ascorbic acid content does not vary as much as had been supposed with maturity.

The values for nicotinic acid and riboflavin vary somewhat between varieties but not to as great an extent as the other vitamins. In the case of nicotinic acid the highest value is 60 per cent greater than the lowest, and in the case of riboflavin the highest is 50 per cent greater.

The results (Table 2) are similar to those given in Table 1, except that there are only two vitamins listed. The results are even more variable, probably because of the greater number of varieties.

The vitamin contents of inbred lines of golden sweet corn together with hybrids are shown (Tables 3 and 4); no white sweet corn strains are included. The hybrids consist almost entirely of crosses of inbreds listed in Table 3.

A fairly definite increase in carotene is shown with increasing maturity (lower moisture). Ascorbic acid seems to decrease with increasing maturity. The maturities given represent young and fairly mature corn from the point of view of canning quality. Considerable differences in vitamin are found between different hybrids of the same degree of maturity. Differences are not as marked in the case of ascorbic acid as they are in the case of carotene. It might be noted here that there is no correlation between depth of color and the carotene content, either in the raw or canned corn.

The differences in vitamin content found are of a considerable magnitude and show quite definitely that vitamin content should not be overlooked in breeding better hybrid varieties.

TABLE 2

Vitamin Content of Peas

Culture No.	Carotene	Vitamin C	Culture No.	Carotene	Vitamin C
	γ/gm.	γ/gm.		γ/gm.	γ/gm.
35A	5.10	284	172A	5.04	322
36A	4.89	262	173A	4.63	316
37A	4.68	291	174A	5.00	269
38A	4.60	287	175A	4.82	.....
39A	.....	.....	177A	3.71	242
44A	4.38	300	178A	3.44	223
95A	4.14	316	179A	3.25	215
96A	3.64	286	180A	3.20	208
97A	3.22	192	181A	3.90	236
98A	3.65	329	182A	3.69	225
103A	3.20	277	183A	3.44	232
105A	3.26	226	184A	2.91	222
106A	3.64	259	186A	2.43	268
107A	3.17	261	187A	3.03	274
109A	3.19	216	188A	2.74	244
110A	3.70	226	189A	3.12	269
112A	3.57	235	190A	2.72	229
113A	4.29	239	191A	2.29	277
114A	3.15	219	192A	3.27	253
116A	3.98	251	193A	2.74	253
128A	3.97	249	194A	3.13	232
140A	3.54	232	195A	2.63	250
142A	3.50	225	196A	2.66	252
144A	3.57	254	197A	3.13	265
145A	3.33	233	198A	2.62	251
147A	3.86	268	199A	3.14	266
152A	3.66	248	200A	3.06	221
154A	4.41	275	201A	3.24	246
155A	1.25	.....	202A	3.50	237
158A	5.24	294	203A	3.57	259
159A	1.09	.....	204A	3.01	243
160A	4.42	244	205A	3.03	225
162A	4.10	272	206A	2.31	220
165A	5.39	289	207A	3.43	285
166A	4.37	257	208A	3.98	232
167A	4.35	324	209A	3.29	236
168A	5.28	254	211A	3.26	236
169A	4.85	.....	212A	2.91	253
170A	5.32	.....	213A	2.48	234
171A	2.17	.....			

TABLE 3

Vitamin Content of Corn Inbreds

Inbred	Moisture	Carotene	Vitamin C
		γ/gm.	γ/gm.
1	73.0	2.05	149
	66.0	2.49	.....
2	69.0	2.47	154
	63.5	2.85	150
3	68.0	0.98	192
	65.0	1.20	216
4	73.0	1.86	151
	68.0	2.63	139
5	69.0	1.37	162
	64.0	3.32	123
6	72.0	2.17	.....
	69.0	2.80	167
7	72.0	2.17	116
	67.0	4.43	123
8	69.0	4.14	128
	66.0	4.87	128
9	73.0	2.51	175
	65.0	3.13	146
10	70.5	0.81	138
	66.0	1.22	114
11	71.0	4.02	165
	67.0	4.11	167
12	70.0	2.05	189
	67.5	2.10	.....
13	74.0	1.18	142
	71.0	2.05	152
14	71.0	2.11	190
	65.0	3.14	194
15	74.0	1.02	128
	69.0	1.47	107
16	73.0	2.01	174
	66.0	2.34	158
17	71.0	1.54	184
	66.0	1.75	204
18	76.0	2.08	183
	69.5	3.88	128
19	74.0	1.38	195
	64.0	1.68	182
20	70.0	6.13	104
	66.5	7.73	105
21	76.0	2.23	230
	67.0	2.50	167
22	72.0	4.40	270
	67.0	4.82	238

Differences such as are found in the above tables make a considerable difference in the nutritional value of these vegetables. Perhaps too much emphasis in breeding programs in the past has been placed on such factors as yield, flavor, tenderness, and the like, and too little on nutritional quality. More emphasis will have to be placed on this in the future.

This present article emphasizes the differences found in various lines of peas and corn. These lines were chosen for vitamin study because all

TABLE 4

Vitamin Content of Corn Hybrids

Hybrid	Moisture	Carotene	Vitamin C
		γ/gm.	γ/gm.
1	70	1.65	129
2	72	2.19	142
3	71	1.97	128
4	73	2.77	125
5	72	2.79	113
6	73	3.69	102
7	72	3.06	156
8	71	2.89	131
9	70	2.42	161
10	71	2.75	158
11	71	3.22	154
12	73	4.27	116
13	72	3.36	99
14	71	3.19	165
15	70	4.50	131
16	73	3.84	138
17	73	2.47	129
18	70	3.71	118
19	70	3.85	120
20	73	3.75	149
21	72	3.44	128
22	72	1.68	143
23	72	2.96	149
24	72	2.70	146
25	72	3.10	152
26	72	2.37	122
27	72	1.88	140
28	73	3.10	122
29	72	3.07	117
30	72	2.98	149
31	70	3.81	141
32	72	3.26	151
33	73	2.73	118
34	70	3.12	136
35	75	3.42	124
36	72	3.20	143
37	74	2.31	155
38	72	3.77	180
39	72	2.39	176
40	72	2.32	145
41	72	3.32	163
42	73	3.42	120
43	72	4.60	178
44	71	3.60	164
45	72	2.76	101

show some degree of merit for commercial canning. Most of these will be eliminated. Vitamin content will be considered among the qualities which make a particular line desirable.

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A SUGGESTION FOR THE RAPID PRESUMPTIVE EXAMINATION OF FOODS SUSPECTED OF HAVING CAUSED STAPHYLOCOCCAL FOOD POISONING

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A major problem occurs in bacteriologic investigations of suspected staphylococcal food poisoning when the organisms are no longer viable at the time the food is examined in the laboratory (e.g., when there has been excessive delay in delivering the food to the laboratory). Another bacteriologic disadvantage is that most methods of examination require several days, particularly when animal inoculation experiments are made, and can be applied to only a small number of samples. Hence, a method which takes only an hour or so and does not require that the staphylococci be viable should be of distinct advantage in epidemiologic investigations. Moreover, if the test is sufficiently simple, a large number of samples can be examined with little effort.

Search of the literature shows (1) that when the coagulating power of food-poisoning staphylococci was reported, the reaction was positive in all but one instance—in fact, Dienst (1942) suggested that the method may prove valuable as an exclusion test; and (2) that, with rare exceptions, the organisms were present in the foods in large numbers, in relatively pure culture.

Although other pathogenic staphylococci also clot plasma, the presence of plasma-clotting staphylococci in a suspected food would certainly point suspicion to that food and would warrant further study of it. Since the test could be made in as short as an hour by the method to be described, this preliminary investigation would not seriously retard other bacteriologic investigations and might assist in the selection of foods for such studies.

Since the coagulating principle is present in cultures of staphylococci, it should be present in foods that have supported considerable growth of staphylococci, even though the organisms are no longer viable. Investigation of a small number of suspected foods in this laboratory confirms this. Therefore, the suggestion is offered that the coagulation reaction be applied directly to foods to be tested by emulsifying a loopful or more in 0.5 ml. of Bacto tryptose phosphate broth (which enhances the clotting power), adding 0.5 ml. of citrated or oxalated rabbit plasma or human citrated or oxalated *whole blood*, and incubating up to seven hours, examining hourly. This prolonged incubation is desirable to permit growth of pathogenic staphylococci in case the amount of coagulating principle should have been inadequate in the original food.

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EFFECT OF MODIFIED ATMOSPHERE STORAGE ON ASCORBIC ACID CONTENT OF SOME VEGETABLES[1]

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Many investigators have demonstrated that the ascorbic acid content of fresh vegetables decreases rapidly after harvest. It has been estimated that some vegetables shipped without icing or refrigeration lose as much as one-half of their original content of ascorbic acid before they reach the ultimate consumer. The rate at which this loss occurs varies with different vegetables; it is relatively slow in peppers, tomatoes, cabbage, and root crops but rapid in leafy vegetables.

The loss of ascorbic acid from plant tissue after harvest is essentially an oxidation process. It is logical to assume, therefore, that any treatment which reduces the rate of oxidation processes in plant material also inhibits the destruction of ascorbic acid. Having demonstrated that the presence of carbon dioxide in the storage atmosphere markedly depresses the respiration rate of asparagus, Thornton (1937) investigated the rate of ascorbic acid losses in asparagus held in an atmosphere in which varying proportions of nitrogen had been replaced by carbon dioxide. Contrary to expectations, Thornton observed that the presence of carbon dioxide in the storage atmosphere actually hastens the destruction of ascorbic acid. In offering an explanation for this effect, he points out that the pH of asparagus tissue increases with rising concentrations of carbon dioxide in the air. As is generally known, a high degree of acidity, that is, a low pH, has a stabilizing effect on ascorbic acid.

With the development of methods of modified atmosphere storage for fruits and vegetables it has been recognized that a lowering of the oxygen content of the air is equally, if not more, effective in inhibiting respiration and other oxidation processes than the presence of carbon dioxide. Platenius (1943) showed, for instance, that the respiration of some vegetables can be reduced to one-half of the normal rate by lowering the oxygen content of the air to a level of two to five per cent.

The aim of this study was to find out whether a low oxygen content of the storage atmosphere has the same inhibiting effect on the destruction of ascorbic acid as it has on respiration and other metabolic processes. In some experiments, part of the oxygen was replaced by carbon dioxide in order to determine the effect that the presence of carbon dioxide in combination with a low level of oxygen has on the loss of ascorbic acid of various plant tissues during the storage.

An experiment by Tressler and Moyer (1941) suggests that a beneficial effect of modified atmosphere storage on the ascorbic acid content of plant

[1] Paper No. 261, Department of Vegetable Crops, Cornell University, Ithaca, N. Y.

tissue can be expected. Bartlett pears were stored in five-gallon tin cans provided with an air vent $\frac{5}{64}$ inch in diameter. These fruits retained as much ascorbic acid in seven months as did a corresponding lot kept in the open for three months. The average carbon dioxide content within the cans was three to four per cent, the oxygen concentration 18 to 19 per cent.

MATERIALS AND METHODS

The vegetables used in this study were asparagus (*Asparagus officinalis* L.), sprouting broccoli (*Brassica oleracea*, var. *italica*), snap beans (*Phaseolus vulgaris*), kale (*Brassica oleracea*, var. *acephala*), Brussels sprouts (*Brassica oleracea*, var. *gemmifera*), peas (*Pisum sativum* L.), and spinach (*Spinacia oleracea* L.). All vegetables were grown in the gardens of the Department of Vegetable Crops at Cornell University, and the experiments were started within two hours after harvest.

Depending on the size of individual specimens each lot was divided into samples of from 100 to 500 grams. Great care was taken to have each sample contain an equal number of specimens which were as uniform in size and maturity as could be selected. No sample contained less than 25 specimens.

All experiments were carried out in constant temperature rooms where fluctuations in temperature did not exceed two degrees Centigrade. Except for brief periods when samples were taken for gas analysis, the vegetables were kept in the dark. Wide-mouth glass jars with tightly fitting lids were used as storage chambers.

In those experiments in which the storage atmosphere consisted only of nitrogen and oxygen, the carbon dioxide produced during respiration was absorbed by a 2 N solution of sodium hydroxide in the bottom of the jar. A constant concentration of oxygen was maintained by connecting the jar with a glass cylinder containing oxygen under atmospheric pressure. This cylinder in turn was connected to a Mariotte bottle which replaced the oxygen in the cylinder with water at the same rate as oxygen was utilized by the respiring tissue. A detailed description of the apparatus has been given by Platenius (1942) in an earlier paper. At the beginning of each experiment the jar was flushed with nitrogen from a storage cylinder long enough to reduce the oxygen content to the level desired. The exact composition was then determined by means of a Hays gas analyzer. Each day the gas composition was checked and it was found that the oxygen concentration of the atmosphere could be kept well within a range of 0.3 per cent for the duration of each experiment.

A slightly different procedure was used in those experiments in which the storage atmosphere consisted of a mixture of nitrogen, oxygen, and carbon dioxide. First, the jars were flushed with nitrogen and subsequently with carbon dioxide until the composition of the gases was approximately as desired. The exact composition of the storage atmosphere was ascertained by gas analysis. Stopcocks attached to the lid of the jar were then closed. Twice each day the composition of the atmosphere was readjusted by flushing with nitrogen and oxygen. Because of the large volume of gas in comparison with the plant material in the chamber, changes in the composition of the atmosphere were small and could be maintained well within one-half of one per cent throughout the experiment.

At the beginning and end of each experiment total ascorbic acid was determined in aliquot samples. The method of analysis was essentially the same as that devised by Bessey and King (1933). Triplicate samples were extracted with a mixture of 1 N sulfuric acid and two per cent metaphosphoric acid. A Waring blender served to macerate the tissue. Titrations were made against dichlorophenolindophenol which was standardized against pure ascorbic acid. By weighing each lot at the beginning and end of the experiments, it was possible to express the results on the basis of the original fresh weight.

RESULTS AND DISCUSSION

Asparagus: The data from six storage experiments with asparagus (Table 1) show a definite, inverse relationship between the percentage of oxygen in the atmosphere and the ascorbic acid content of the stored samples. In the first experiment carried out in 1941 the control lot re-

TABLE 1

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Asparagus

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
June 1941	20	0	.....	.404	.404	100.0
		3	Normal air	.108	.105	26.0
		3	10.2% O_2	.149	.145	35.9
		3	2.0% O_2	.276	.272	67.3
May 1942	24	0	.....	.501	.501	100.0
		2	Normal air	.122	.118	23.6
		2	10.2% O_2	.165	.155	30.9
		2	10.0% O_2–10.1% CO_2	.116	.114	22.8
		2	4.5%O_2	.230	.221	44.1
		2	4.5%O_2–17.3% CO_2	.139	.137	27.3
June 1942	23	0	.....	.443	.443	100.0
		3	Normal air	.102	.096	21.7
		3	2.4% O_2	.300	.285	64.3
		3	2.2% O_2– 5.4% CO_2	.285	.280	63.2
		3	2.2% O_2–18.6% CO_2	.255	.247	55.8
June 1941	10	0	.....	.477	.477	100.0
		8	Normal air	.109	.106	22.2
		8	1.2% O_2	.257	.253	53.0
May 1942	10	0	.....	.454	.454	100.0
		7	Normal air	.126	.121	26.7
		7	2.1% O_2	.187	.180	39.6
		7	2.0% O_2– 5.4% CO_2	.207	.203	44.7
June 1942	10	0	.....	.414	.414	100.0
		7	Normal air	.108	.093	22.5
		7	1.6% O_2	.149	.146	35.3
		7	1.7% O_2–17.9% CO_2	.164	.159	38.4

tained only 26 per cent of its original ascorbic acid content while the sample held in an atmosphere containing two per cent oxygen retained 67.3 per cent, that is 2.6 times as much ascorbic acid as the lot in normal air. Essentially the same results were obtained when the experiments were repeated in 1942.

The storage temperature had little influence on the effect of oxygen concentration. Experiments carried out at a temperature of 10°C.(50°F.) led to the same general conclusions as those obtained at room temperature. It was evident, however, that at a given oxygen concentration the power to inhibit the destruction of ascorbic acid was smaller at the lower than at the higher temperature.

TABLE 2

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Peas

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
July 1942	24	0	.....	.291	.291	100.0
		3	Normal air	.202	.191	65.6
		3	2.5% O_2	.280	.268	92.0
		3	2.3% O_2–19.5% CO_2	.257	.249	85.6
July 1942	10	0	.....	.346	.346	100.0
		8	Normal air	.269	.262	75.7
		8	2.2% O_2	.291	.283	81.8
		8	2.3% O_2–18.7% CO_2	.248	.241	69.7

Adding carbon dioxide to the storage atmosphere produced different results at low and high temperatures. At room temperature the presence of carbon dioxide caused more ascorbic acid destruction than was observed in comparable samples kept at about the same oxygen concentration in the absence of carbon dioxide. These results are in agreement with the findings of Thornton (1937). It must be remembered, however, that Thornton's experiments differed from the present ones in that the oxygen concentration of the atmosphere was maintained at a normal level.

The effect of carbon dioxide in the air was strikingly different when asparagus was stored at a temperature of 10°C. In both experiments more ascorbic acid was retained in the presence than in the absence of carbon dioxide. Apparently a change in temperature completely reverses the effect of carbon dioxide on the ascorbic acid content of the samples. As will be shown later, this apparent inconsistency was observed in other vegetables.

Peas: The results of two experiments with peas are presented (Table 2); a comparison of these data with those of asparagus shows that the initial ascorbic acid content of peas is much smaller than that of asparagus. On the other hand, the vitamin is more stable in peas than in asparagus. A low oxygen concentration of the atmosphere reduced the loss of ascorbic acid and the presence of carbon dioxide in concentrations of about 19 per cent accelerated losses at both temperatures, 24 and 10°C.(75.2 and 50°F.).

Snap Beans: A single storage experiment with snap beans, in which the oxygen level was the only variable, gave essentially the same results as the studies with asparagus and peas. At an oxygen concentration of 1.2 per cent the rate of ascorbic acid losses was only one-half as great as in air of normal composition (Table 3).

TABLE 3

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Snap Beans

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
August 1941	20	0	.....	.200	.200	100.0
		4	Normal air	.047	.046	23.0
		4	4.8% O_2	.052	.051	25.5
		4	1.2% O_2	.094	.093	46.5

Spinach: The response of spinach to modified atmosphere was similar to that of asparagus (Table 4). Regardless of the storage temperature, samples held in an atmosphere of low oxygen content retained more than three times as much ascorbic acid as the control lots in normal air. The presence of 18 per cent carbon dioxide in the air accelerated the destruction of ascorbic acid at 20°C.(68°F.) while at 10°C. the addition of 5.3 per cent carbon dioxide slightly retarded its loss.

TABLE 4

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Spinach

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
June 1942	24	0	.....	.729	.729	100.0
		3	Normal air	.130	.124	17.0
		3	1.2% O_2	.420	.393	53.9
		3	1.2% O_2–17.9% CO_2	.290	.267	36.6
June 1942	10	0	.....	.593	.593	100.0
		11	Normal air	.059	.053	8.9
		11	1.5% O_2	.211	.190	32.0
		11	1.5% O_2– 5.3% CO_2	.219	.214	36.1

Kale: Kale had the highest initial ascorbic acid content of any of the vegetables included in these experiments (Table 5). At a temperature of 20°C. and an oxygen level of 3.1 per cent more than three times as much ascorbic acid was retained during storage than was retained in the control lot. Reducing the oxygen to still lower levels caused a marked rise in the rate of destruction. At a concentration of 0.8 per cent losses were almost as high as in normal air. An examination of this particular

sample revealed that the tissue had been injured severely by suboxidation. A pronounced alcoholic odor was noticed when the chamber was opened. Probably the presence of alcohol or other end products of anaerobic fermentation was detrimental to the vitamin.

TABLE 5

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Kale

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
October 1941	20	0	.....	1.884	1.884	100.0
		3	Normal air	0.570	0.561	29.8
		3	3.1% O_2	1.757	1.749	92.8
		3	0.8% O_2	0.583	0.578	30.7
		3	6.3% O_2–12.7% CO_2	1.683	1.668	88.5

Whether or not the rate of ascorbic acid losses are affected by the presence of carbon dioxide cannot be ascertained from the data since none of the samples was held at a comparable oxygen level. At any rate, losses in ascorbic acid in an atmosphere containing 6.3 per cent oxygen and 12.7 per cent carbon dioxide were only slightly higher than in samples held at 3.1 per cent oxygen. This suggests that even at a temperature of 20°C. the effect of carbon dioxide is beneficial instead of detrimental.

TABLE 6

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Sprouting Broccoli

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
August 1942	24	0	.....	1.419	1.419	100.0
		3	Normal air	0.697	0.551	38.8
		3	2.2% O_2	1.136	0.976	68.8
		3	2.2% O_2–17.0% CO_2	1.268	1.174	82.7
August 1942	10	0	.....	1.258	1.258	100.0
		6	Normal air	0.856	0.714	56.8
		6	1.5% O_2	1.081	0.865	68.8
		6	1.7% O_2–16.6% CO_2	1.255	1.148	91.3

Broccoli: The results of two storage experiments with broccoli (Table 6) show that the benefit from low oxygen storage was small. At a temperature of 10°C., especially, only little more ascorbic acid was retained at a reduced oxygen concentration than in the control sample. At both temperatures, 24 and 10°C., the addition of carbon dioxide in concentrations of about 17 per cent had a decided beneficial effect.

Brussels Sprouts: The data for Brussels sprouts (Table 7) were similar to those for broccoli. The beneficial effect of a low oxygen level was smaller than in any of the other vegetables examined. This may be due in part to the fact that the level of oxygen was slightly higher in these experiments than in others. As in broccoli, carbon dioxide in the storage atmosphere somewhat retarded losses of ascorbic acid at both temperatures.

TABLE 7

Effect of Modified Atmosphere Storage on Ascorbic Acid Content of Brussels Sprouts

Time of experiment	Storage temp.	Days stored	Modification of storage atmosphere	Ascorbic acid		
				Mg. per gm.	Mg. per gm. of orig. fresh wt.	Orig. retained
	°C.					pct.
October 1942	24	0	.....	1.095	1.095	100.0
		4	Normal air	0.849	0.604	55.2
		4	2.6% O_2	1.046	0.748	68.3
		4	2.6% O_2–18.4% CO_2	1.023	0.919	83.9
October 1942	10	0	.....	1.498	1.498	100.0
		10	Normal air	1.239	1.079	72.0
		10	2.3% O_2	1.422	1.226	81.8
		10	2.5% O_2–17.2% CO_2	1.525	1.328	88.7

SUMMARY

The effect of modified atmosphere storage on the rate of ascorbic acid destruction of seven vegetables was studied at temperature levels varying from 10 to 24°C. (50 to 75.2°F.).

A reduction of the oxygen content of the storage atmosphere always resulted in a lowering of the rate at which ascorbic acid was lost. The effectiveness of the treatment varied with different vegetables, with the level of oxygen, and the temperature of the storage room. Asparagus, spinach, and kale gave the best response to modified atmosphere storage. At oxygen concentrations below four per cent the retention of ascorbic acid in these vegetables was 2.6 to 4 times as high as in comparable samples stored in normal air. The data indicate that at a given oxygen level the effectiveness of the treatment decreases somewhat with a lowering of the temperature.

Inconsistent results were obtained when carbon dioxide in concentrations as high as 20 per cent was added to the atmosphere containing small proportions of oxygen. In broccoli and Brussels sprouts the presence of carbon dioxide further inhibited losses of ascorbic acid regardless of the temperature at which the experiments were carried out. The presence of carbon dioxide accelerated the destruction of ascorbic acid in asparagus and spinach at room temperature but retarded it at a temperature of 10°C. In peas the presence of carbon dioxide had a detrimental effect on the vitamin content at both temperature levels.

The data furnish additional evidence for the statement that vegetables held in cold storage, common storage, or outdoor bins require only a minimum of ventilation. At temperatures commonly employed for the storage

of vegetables a lowered oxygen concentration and increased carbon dioxide content of the air has a beneficial instead of detrimental effect. Ventilation for vegetables in storage is needed only to remove heat during the beginning of the storage period and to prevent the complete utilization of oxygen by respiration.

Whether or not the storage of vegetables under controlled, modified atmosphere will find commercial application is doubtful. Although the storage in modified atmosphere materially retards the loss of flavor and food value of vegetables, the cost of constructing gas-tight storage rooms and of maintaining the desired gas composition may prove to be too high to justify its commercial application. It also must be remembered that the spoilage of vegetables in storage is caused primarily by the action of molds and bacteria, the development of which is little affected by modification of the storage atmosphere.

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ASCORBIC ACID CONTENT OF TOMATOES

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Since tomatoes are one of the commonly available sources of ascorbic acid, we thought that an investigation of some of the factors which affect the concentration of the vitamin in this fruit would yield interesting results.

It has been shown by numerous investigators that the ascorbic acid content of fruits and vegetables may vary under certain conditions. Among the factors reported by various investigators as being responsible for these fluctuations are the following: conditions of ripening by House, Nelson, and Haber (1929), Wokes and Organ (1943), Kohman and Porter (1940), Hamner and Maynard (1942), and Harris (1941); soil and fertilizer by Suguira (1938), Tressler, Mack, and King (1936), Von Hahn and Görbing (1933), Ijdo (1936), Isgur and Fellers (1937), Potter and Overholzer (1933), Wachholder and Nehring (1938), Hamner, Lyon, and Hamner (1942), and Hester (1941); maturity by Bessey (1938), Oliver (1938), Wasson (1931), Brown and Moser (1941); and processing by Tressler and Curran (1938), Floyd and Fraps (1942), and Mituda (1938). The effect of the variety of the fruit and conditions of soil are well established by Maclinn, Fellers, and Buck (1937), Yarbrough and Satterfield (1939), Currence (1940), and Gomolyako (1937). We thought it advisable, therefore, to confine our studies to one variety at the same location, and to determine the effect of rainfall and sunshine on the vitamin C content of the fruit.

This study of Illinois-grown tomatoes was begun in 1942 and continued during the 1943 season.

EXPERIMENTAL PROCEDURE

Nature and Source of Samples: The tomatoes tested were of the early Baltimore variety. They were grown at the Cook County Experiment Station of the University of Illinois, College of Agriculture, located at DesPlaines, Illinois.

The 1942 series were planted in the greenhouse on April 2, shifted to a soil bed on April 17, and transplanted out of doors on May 20, in a Lisbon clay loam to which 500 pounds per acre of 4-8-6 fertilizer was applied on April 8, 1942. The 1943 samples were collected from plants which were planted in the greenhouse on March 20, shifted to the bench on April 16, and transplanted to the field on May 26. The fertilizer on the plot consisted of 400 pounds of a 4-12-6 mixture per acre.

On July 8 and 22, 1942, the first or second and third blossoms of the inflorescence were tagged if the flowers appeared of approximately the same age. The tagging for the 1943 series was done on July 17, 1943. Using

[1] Part of experimental data in this paper are taken from a thesis submitted by Ignatius J. Kaski in partial fulfillment of the requirement for the degree of Master of Science in Chemistry in the Graduate School of the University of Illinois.

the fruit which had developed from these tagged blossoms, we assumed that the samples collected at any particular time were approximately the same age.

Analytical Procedure: The samples were picked at the intervals shown (Table 1). Quarters of several tomatoes were taken to make up a composite sample of 100 grams. These samples were weighed directly into 50 ml. of 10-per cent metaphosphoric acid and mixed for about two minutes in a Waring blender. The mixture was then transferred to a 250-ml. volumetric flask and made up to volume with distilled water. Either five or 10 ml. of filtrates were titrated according to the method of Bessey and King (1933).

Several of the filtrates were treated with hydrogen sulfide, as described by Floyd and Fraps (1942), while others were treated with ascorbic acid oxidase according to the procedures of Tauber and Kleiner (1935) and Kraemer and Satterfield (1942). Since the hydrogen sulfide treatment did not cause any increase in the titratable ascorbic acid, it was evident that the tomatoes did not contain any reversibly oxidized ascorbic acid and since, after the oxidase treatment, the titration values were practically zero, it was apparent that substances interfering with the reaction were absent. Both procedures were omitted after the first few determinations.

DISCUSSION

Our results on the ascorbic acid content of tomatoes are in agreement with the averages reported in the literature. In following the vitamin C content of the growing fruit from blossom to maturity, we observed rather large fluctuations in the titration values for samples collected on different days. The variations occurred in the green and ripe fruit. A possible explanation for these fluctuations in the vitamin C values can be obtained from a study of the weather data on rainfall and sunshine during the experimental periods. It appears that low ascorbic acid values were found when the fruit was picked on rainy or cloudy days and that the high values were obtained at intervals when a high percentage of possible sunshine preceded the collection of the samples. This is in agreement with the findings of Wokes and Organ (1943) who concluded from the results of their experiments that exposure to sunshine during the ripening may have a favorable effect on the vitamin C content of tomatoes.

We could find no significant differences in ascorbic acid values in green and ripe tomatoes collected at the same time. This observation was made also by Wokes and Organ (1943) and Maclinn and Fellers (1938).

The possible influence of the water content and the pH of the pulped fruit on the vitamin C content was considered and investigated on the series of 1943. No close correlation between these factors and the titration values could be observed (Table 1).

Reid (1938, 1943) has pointed out that some plants lose vitamin C during periods of darkness at a more rapid rate than it is synthesized. She observed net losses as high as 20 per cent during the night and net increases up to 25 per cent during bright days. In view of these observations, it might be predicted that a cloudy or rainy day would be marked by a lower production of ascorbic acid than days of bright sunshine. Our results indicated that this was true in the case of tomatoes (Figs. 1 to 3).

TABLE 1

Ascorbic Acid Content of Tomatoes

Age of fruit [1]	Series I 1942	Series II 1942	Series of 1943		
	Mg. of ascorbic acid	Mg. of ascorbic acid	Mg. of ascorbic acid	pH of pulped fruit	Water content of fruit
days	*pct.*	*pct.*	*pct.*		*pct.*
14	23.17	32.69	24.05	5.00	92.44
21	18.12	23.91	17.19	4.87	93.58
28	17.36	.....	16.07	4.79	94.84
29	.....	25.57	.....	.....	.....
35	18.68	.....	21.15	4.39	90.26
36	.....	28.00	.....	.....	.....
40	.....	.....	19.68	4.18	93.18
43	33.26	25.16	.....	.....	.....
45	.....	.....	23.09	4.18	93.60
47	.....	.....	19.34	4.18	94.48
48	28.36	.....	.....	.....	.....
49	.....	22.65	24.88	4.23	94.25
50	28.18	.....	.....	.....	.....
52	.....	.....	22.20	4.30	94.58
54	.....	20.62	.....	.....	.....
57	26.39	.....	.....	.....	.....

[1] Time in days counted from the day the blossoms were tagged to the day the fruit was picked.

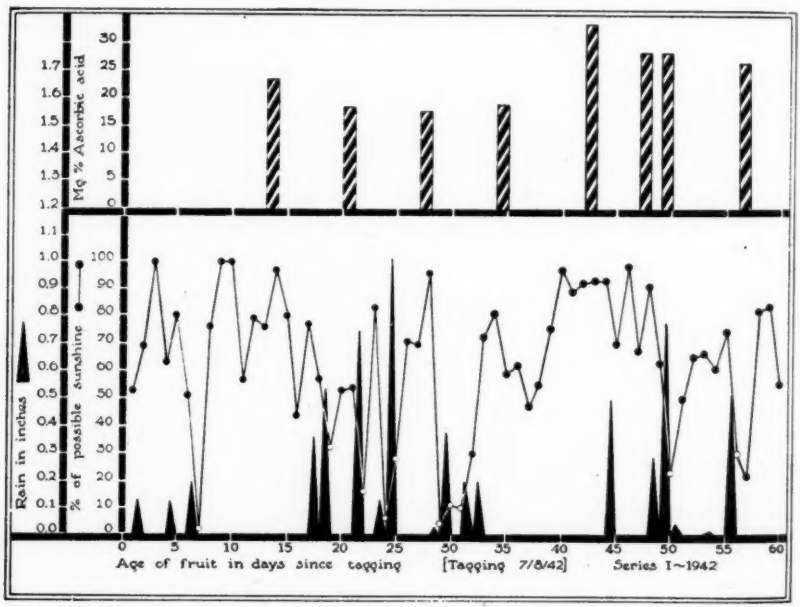

FIG. 1.

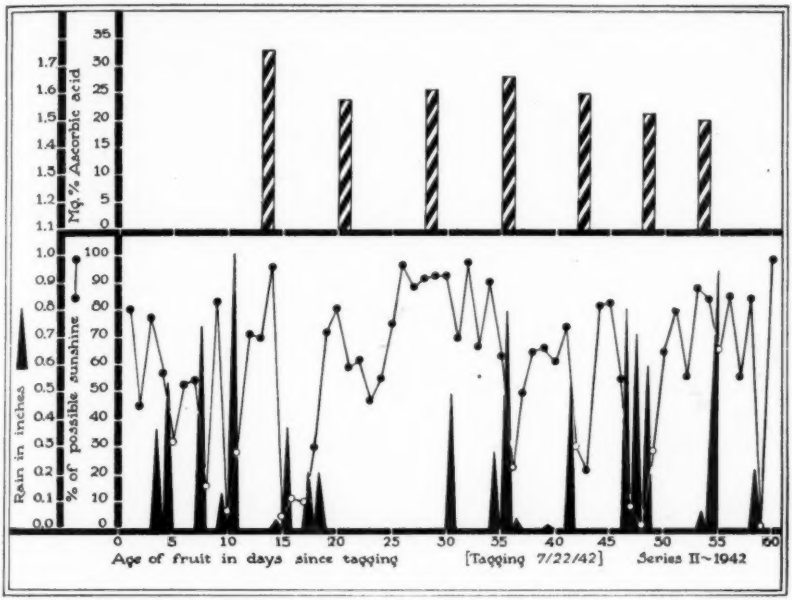
Mg. % Ascorbic acid
% of possible sunshine
Rain in inches
Age of fruit in days since tagging
[Tagging 7/22/42]
Series II~1942

FIG. 2.

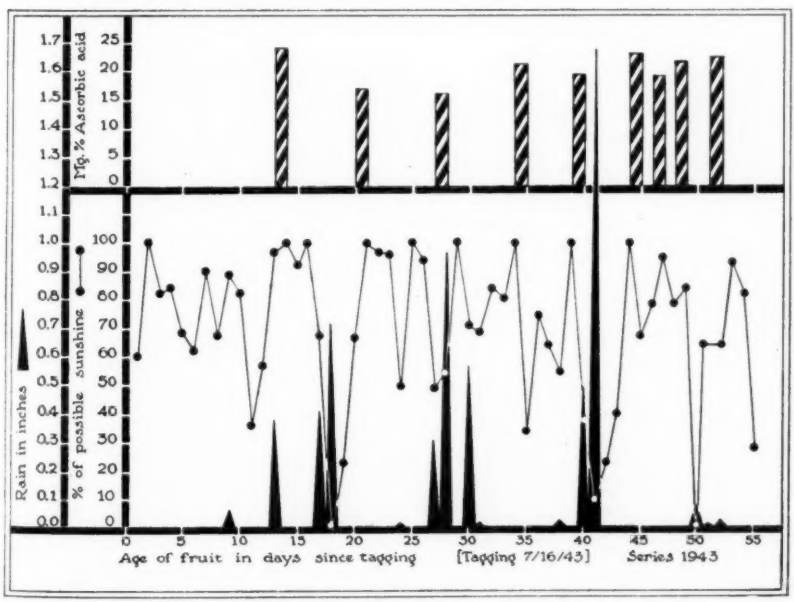
Mg. % Ascorbic acid
% of possible sunshine
Rain in inches
Age of fruit in days since tagging
[Tagging 7/16/43]
Series 1943

FIG. 3.

Since all the plants from which these fruits were picked had been grown in the same plot, one may assume that rain and sunshine were the variables affecting the vitamin C content. It will be noted that the titration values for the 14-day fruit of Series II, 1942, are higher than those obtained for the fruit of the same age of the other two series. The rainfall was greater during the first two weeks of the growth period for Series II, 1942, than it was during the comparable period for the other series. It would seem that abundant moisture has a favorable influence on the development of ascorbic acid in the very young fruit. The effect of sunshine and rainfall during the subsequent period of growth and ripening seems to be different. Long hours of sunshine have a beneficial influence on the ascorbic acid balance of the fruit, while tomatoes collected shortly after a rain showed a decrease in their vitamin C content. These fluctuations occurred in all three of these series. It would seem, therefore, that in order to insure the highest possible amount of ascorbic acid in tomatoes, it would be desirable to collect the fruit after an interval of some sunshine, climatic conditions permitting, and if it is economical to do so.

SUMMARY

Three series of tomatoes of early Baltimore variety were tested for their ascorbic acid content during various stages of development.

An analysis of the available weather data provides a basis for the assumption that sunlight and rainfall may be the causal agents for the variations in ascorbic acid content.

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DETERMINATION OF ESTERS IN WINES—LIQUID-LIQUID EXTRACTION

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In the present edition of *Methods of Analysis* of the A.O.A.C. there is neither a tentative nor official method for the determination of esters in wines. The distillation method prescribed for distilled liquors has been used for wines, Nelson and Wheeler (1939), but it only indicates volatile neutral esters and these only approximately with wines. The percentage of the esters determined by distillation procedures depends on the composition of the wine and the design and rate of operation of the distilling apparatus. The ester content of distilled liquors is practically all volatile esters; wines have nonvolatile and volatile neutral esters as well as relatively nonvolatile acid esters. The ester content of wines is extremely variable since the various types of wines vary markedly in pH, acidity, alcohol content, and microorganism population. Ethyl lactate occurs in spoiled wines and, since it is less volatile than ethyl acetate, misleading results for the volatile esters may be obtained by distillation procedures. Distillation procedures, however, are capable of giving approximate results for the ethyl-acetate content of wines.[1] Direct saponification, although used by many early enologists, is impracticable since tannins, sugar, and other substances present in wines also consume alkali.

Liquid-liquid extraction of esters has been used very little for wines. Berthelot (1899) used ethyl ether, shaking neutralized wine with the ether and then saponifying. His unfamiliarity with the partition coefficients undoubtedly led to low results. Scurti (1910) also used ethyl ether for the extraction and qualitative identification of the esters. Since ethyl ether also extracts tannins and other saponifiable substances, petroleum ether was suggested by Espil and Peynaud (1936). In comparative tests Peynaud (1937) found only one-fourth as much apparent esters by petrol-ether extraction compared with ethyl-ether extraction. The method used in the present study is essentially that of Peynaud.

EXPERIMENTAL PROCEDURE

Apparatus: A simple extraction apparatus (Fig. 1) was constructed of Pyrex glass for the experiments. The central tube, about 12 inches in length and 1½ inches in diameter, had a side arm placed at a height of four inches. The inner tube, about ½ inch in diameter, had holes about 1/36 inch in diameter in the bottom. The central tube was connected to a water-cooled condenser at the top. The side arm was attached to a

[1] Several short methods for determining the approximate neutral ester content of wines have been tried in this laboratory. Archinard's (1939) method gave 53 per cent recovery. Peynaud has published two short procedures; his original method (1937) gave 75.5 per cent recovery from ethyl acetate solutions. Wines to which known amounts of ethyl acetate were added gave a 73.5 per cent recovery. Peynaud's revised procedure (1939) is somewhat like Archinard's. The recovery from synthetic solutions was 58 per cent. Grandchamp and Vollaire-Salva's method (1939) has not been tried.

250-ml. Erlenmeyer flask. Neoprene stoppers were used, since rubber is not advisable with warm petrol-ether systems. The Erlenmeyer flask was placed in a hot-water bath, the temperature of which was thermostatically controlled by submerged electric heaters.

The distillation apparatus was a 500-ml. Pyrex ammonia type with a Graham condenser (Corning No. 3340).

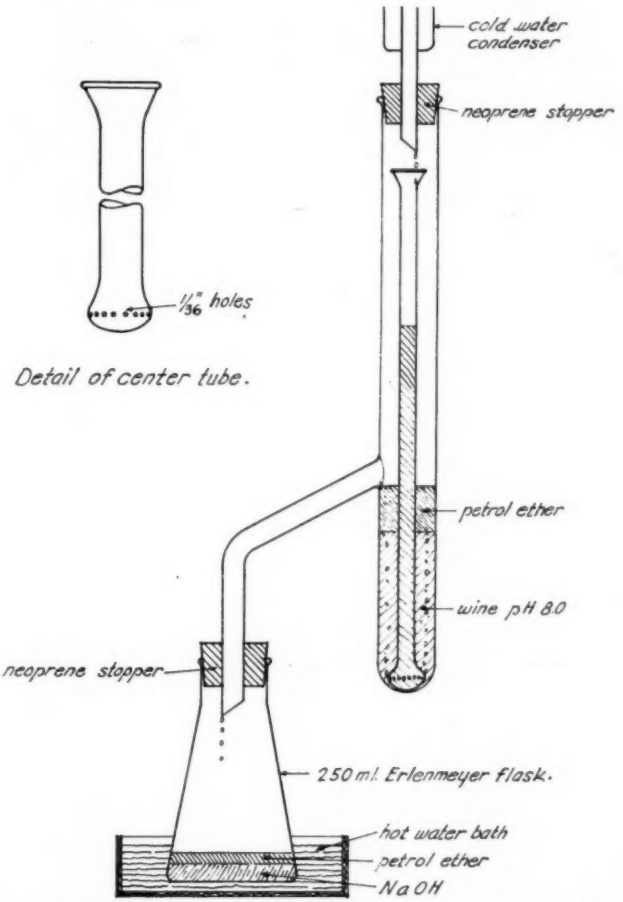

FIG. 1. Apparatus for the liquid-liquid extraction of esters from wines.

Reagents: Petrol ether was redistilled over sodium hydroxide and the fractions boiling below about 65°C.(149°F.) were saved. Carbonate-free 0.0333 *N* sodium hydroxide was used for the saponification and standardized hydrochloric acid for the back titration. A 10-per cent solution of diglycol laurate (Neutral S of Glyco Products Co., Inc., New York) was prepared before each extraction.

Technic: Twenty-five ml. of wine was carefully neutralized to a pH of 7.5 to 8.0 in a Beckman pH apparatus. The wine was washed into the central tube, two drops of the 10-per cent diglycol laurate added, the inner tube inserted, and the apparatus connected to the water condenser. To the Erlenmeyer flask was added 10 ml. of the 0.0333 *N* NaOH, the sides of the flask were washed down with 50 ml. of carbon

dioxide-free water, and the flask was connected to the side arm. Petrol ether was then poured through the top of the water condenser. The ether falls into the inner tube, and when sufficient head is established, pushes out through the small holes at the bottom. The finer the bubbles rising through the wine the more rapid the extraction of the esters. Sufficient of the ether is poured in until about 100 ml. overflows into the Erlenmeyer flask. The temperature of the water bath was adjusted to about 64°C. (147.2°F.) so that approximately 500 ml. of petrol ether was condensed in the condenser per hour. The purpose of the dilute diglycol laurate was to prevent the formation of emulsions as the small bubbles of petrol ether rise through the wine. If emulsions started to form during the extraction, a drop or two more of the diglycol laurate was added.

The extraction was continued for 24 hours. The residual NaOH was titrated with standardized HCl. This titration had to be done very slowly on account of the petrol-ether layer in the Erlenmeyer and the formation of emulsions when the flask was shaken. The amount of NaOH used represents the total neutral esters extracted.

TABLE 1

Neutral Esters in Various Types of California Wines Using a Liquid-Liquid Extraction Method[1]

Wine type	Number of samples	Total	Volatile
Angelica	15	195	85
Tokay	7	342	116
White port	6	188	102
Muscatel	16	224	96
Port	25	277	174
Burgundy	19	357	145
Zinfandel	19	300	113
Sauterne	10	137	65
Dry Sauterne	21	174	120

[1] Milligrams of ethyl acetate per liter.

The neutralized solution was then placed in a separatory funnel, and the neutralized solution run off into a 100-ml. volumetric. The funnel and the petrol ether was washed twice with 15 ml. of carbon dioxide-free water and the washings run into the same volumetric. The solution was brought to volume and placed in the distillation apparatus. Two ml. of N H_2SO_4 was added and eight ml. of water used to wash out the volumetric. One hundred ml. was distilled off, heated to boiling for 20 seconds to rid of carbon dioxide, and titrated with 0.0333 N NaOH. The amount of NaOH used represents about 90 per cent of the volatile neutral esters—ethyl acetate primarily. The milliliters of NaOH used were multiplied by 1.11 and the result subtracted from the amount used in the first saponification. The remainder is the nonvolatile neutral esters. Blank solutions were occasionally extracted and appropriate corrections applied to the titration values. The consumption of alkali by the blanks was very small—less than 0.2 ml. of 0.0333 N NaOH for the total neutral esters and about 0.1 ml. for the volatile esters.

This procedure has been applied to a large number of both table and dessert wines in this laboratory. When the rate of condensation of the petrol ether exceeds 500 ml. per hour, 22 wines extracted for periods up to 30 hours showed little extraction after 25 hours. The pH of the wine, within limits, is apparently not an important factor in the extraction of neutral esters. The pH of samples of two wines was varied from 6.0 to 9.3. Above 9.0 there was some reduction in the total neutral esters extracted.

In 69 commercial, bottled, California dessert wines (Angelica, Muscatel, Tokay, white port, and port) the total neutral esters averaged 247 and the volatile neutral esters 123 as milligrams of ethyl acetate per liter. In 69

commercial, bottled, California table wines (Burgundy, Zinfandel, Sauternes, and dry Sauternes) the total esters averaged 254 and the volatile neutral esters 117 as milligrams of ethyl acetate per liter [2] (Table 1).

Both the total and volatile neutral esters varied tremendously in the various types of wines. Further details of these analyses and their significance will be published later.

SUMMARY

The apparatus and technic of a liquid-liquid extraction procedure for determining the neutral esters in wines is discussed.

The volatile and nonvolatile neutral esters can be approximately separated. Collaborative work on this procedure would be very desirable.

Further work on an extraction method for total esters—neutral esters plus acid esters—is needed.

[2] Liquid-liquid extraction for determining acid esters as well as neutral esters has been tried, but consistent results have not been obtained owing to the difficulty of removing tannins and coloring matter.

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DIRECT MICROSCOPIC METHOD IN BACTERIOLOGICAL CONTROL OF DRIED WHOLE EGGS[1]

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The sudden demand of the British Ministry of Food for enormous quantities of dried whole eggs of a quality suitable for fresh-egg dishes has introduced, among others, the problem of bacteriological control of breaking and drying operations. Since the nine drying plants in Canada are widely scattered, main reliance had to be placed upon bacteriological examination of the powder itself at a central laboratory, supplemented where possible by plant-sanitation surveys.

As a result of discussions between the driers, the British Ministry of Food, and the Special Products Board and its technical advisers the following standards were agreed upon for the 1943 drying season:

Grade A powder (for use in fresh-egg dishes), total viable count on tryptone glucose agar, after 48 hours at 37°C., shall not exceed 500,000 per gram, with *E. coli* absent from 1/100 gram.

Grade B powder (residual portions, for baking purposes only), no viable count limit, but *E. coli* absent from 1/1,000 gram.

While the plate count in particular was of value in directing attention to several cases of faulty plant practice, it was nevertheless noticed that an occasional plant, where sanitary conditions and practices were not above criticism, maintained a surprisingly low level of viable counts. Information at hand [Johns (1943a)] suggested that, as in the case of dried milk, the plate count of egg powder is greatly influenced by the method of drying and of subsequent storage. In the dried-milk field, the direct microscopic method furnishes valuable information concerning the previous history of the product [Prickett (1939)] and it seemed logical to assume that this might also hold true for dried eggs. Bartram (1943a) has recently reported that this is the case. Consequently it was decided to make direct microscopic counts concurrently with plate counts on all samples received after July 26, 1943.

The technique originally employed was to spread 0.01 ml. of a 1/10 dilution of egg powder over an area of one sq. cm. on a clean microscopic slide, then to defat, fix, and stain with the Newman-Lampert combined stain commonly used in milk work. This, however, was not too satisfactory, the background being too dark for easy counting. The acid-methylene blue stain described by Mallman and Churchill (1942) was next tried, but trouble was experienced with films washing off. Finally, the technique described in Standard Methods for the Examination of Dairy Products (1941), using North's aniline oil-methylene blue stain, was adopted with the staining period extended to one to two minutes, as suggested by Bart-

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ram (1943b). This gave much more satisfactory results. We have recently been experimenting with various modifications of Gray's (1943) methylene blue-basic fuchsin stain; this also gives nice results but the technique requires more careful attention than North's. For official control purposes, at least 60 fields should be counted unless organisms are present in very large numbers. As suggested by Bartram (1943b), paired cocci are counted as a single cell to avoid attempting to differentiate between them and long rods with an unstained or lightly stained central spore; where a clump or chain of cells is found, however, each individual cell is counted.

The greater value of the direct microscopic method in indicating the previous history of the product is suggested by the data (Table 1). Plant

TABLE 1

Counts on Whole Egg Powder

Sample		Bacteria count (thousands per gram)		Ratio—D.M.: Plate
		Direct microscopic	Plate	
Plant A	1	330	11	30.0
	2	220	5	44.0
	3	110	12	9.2
	4	110	17	6.5
	5	165	34	4.8
	6	330	23	14.3
	7	110	4	27.5
	8	330	30	11.0
	9	220	10	22.0
Plant B	1	550	8	69.0
	2	330	11	30.0
	3	880	21	42.0
	4	1,100	34	32.0
	5	770	9	86.0
	6	770	9	86.0
	7	440	17	26.0
	8	330	63	5.0
	9	6,200	76	82.0
	10	110	80	1.4
	11	880	91	9.8
	12	660	91	7.3
	13	950	10	95.0
	14	1,300	8	162.0

A, where good sanitary conditions prevailed, showed low counts with both methods. Plant B, where sanitation sometimes suffered as a result of emphasis upon volume of production, showed equally low plate counts (a box-type drier was used) but the direct microscopic counts were almost invariably at a much higher level than in Plant A. That this is not infrequently the case is shown (Fig. 1), wherein the relative counts by direct microscopic and plating methods on a series of 150 prime powders from nine plants are shown.

High counts in the powder may be due either to the use of low-grade eggs or to opportunities for growth of bacteria prior to drying. Since Canadian driers are furnished with high-grade shell eggs containing few

bacteria, Johns (1943b), high counts in Canadian egg powder can almost invariably be attributed to faulty plant practice. The count obtained by the plating method suffers from the disadvantage that it is significantly affected by such factors as the type of drier, drying temperature, speed of removal from drier, rate of cooling, and temperature and length of storage, so that extremely low counts may sometimes be obtained from melange with a moderately high bacterial content. The direct microscopic count, being much less influenced by the factors mentioned, reflects more accurately the conditions under which the melange was handled, and thus appears to be of greater value in control work.

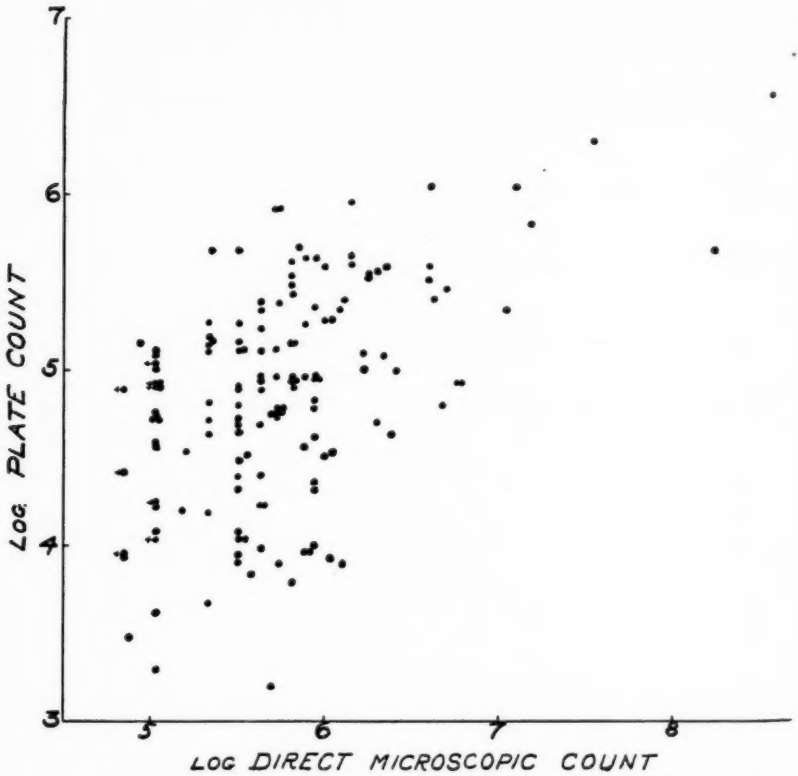

FIG. 1. Comparative counts (expressed as logarithms) of 150 samples of Canadian dried whole eggs by direct microscopic and plate count methods.

In one respect the direct microscopic method as applied to dried eggs is less satisfactory than when applied to dried milk. This is when the melange has been pasteurized before drying. While the degree of heat treatment given egg melange [60°C.(140°F.) for 30 minutes in the present instance] is less severe than that given milk in forewarming, the bacterial cells in pasteurized melange (or powder made therefrom) fail to stain

with either North's or Gray's stains as they do in dried milk. This is illustrated by the counts on samples of pasteurized and unpasteurized melange and powder (Table 2). The plant in question was working with frozen melange of such a high bacterial content that they were experiencing difficulty in meeting the bacteria count limits for Grade A powder. To reduce the count on their powder, vat pasteurization was resorted to with the result that both plate and direct microscopic counts fell to a low level. Similar results have been obtained with powder from a second plant which has followed the same practice of pasteurizing high-count melange. The possibility of so modifying the technique of the direct microscopic method that the organisms destroyed by pasteurization may be rendered stainable is being investigated.

TABLE 2

Comparison of Plate and Direct Microscopic Counts[1] on Pasteurized vs. Unpasteurized Egg

Sample	Melange in thawing tank		Melange in balance tank		Powder	
	D.M.	Plate	D.M.	Plate	D.M.	Plate
Unpasteurized						
Nov. 19	36,000	5,500	330,000	13,000	250,000	240
Nov. 23	46,000	8,900	240,000	15,000	52,000	630
Pasteurized						
Nov. 26	17,000	11,000	110	59	350	12
Dec. 1	3,500	900	220	8	<110	11
Dec. 16	.....	1,600	<110	48	<110	33
Jan. 11	2,900	3,600	75	24	150	16

[1]Given in thousands per gram.

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RIBOFLAVIN AND THIAMIN CONTENTS OF PORK LOIN MUSCLES AND THEIR RETENTION DURING COOKING[1, 2]

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Lane, Johnson, and Williams (1942) and Cheldelein and Williams (1943), in attempting to evaluate the actual amounts of vitamins entering into the average American diet, have made the interesting observation that the wide variations in caloric contributions of different foods to diets often cause the total vitamin contributions of these foods to be quite different from what would be expected on the basis of relative vitamin contents alone. They point out, for example, that white bread, even though a relatively poor source of riboflavin, is second only to milk as a total contributor of riboflavin to the diet. Viewed in this manner, meats are found to contribute about one-fifth of the total riboflavin supply to the diet and one quarter of the thiamin supply. In the meat category, pork is the principal thiamin contributor.

The most extensive investigations of the distribution of vitamins in meats and their retention in cooking have come from the Wisconsin and Texas groups. Mickelsen, Waisman, and Elvehjem (1939) found the liver and kidney of pork, beef, lamb, and veal to be uniformly higher in riboflavin content than other organs of these species. They reported no loss of riboflavin by stewing as contrasted with losses of from 33 to 60 per cent by pan broiling and roasting. McIntire, Schweigert, Henderson, and Elvehjem (1943), in experiments where the cooking conditions were more carefully standardized, reported the average thiamin retention in pork to be 70 per cent after roasting and broiling and 50 per cent after braising. The riboflavin retention averaged 85 per cent with both cooking methods. The total retention in the meat plus drippings was 70 per cent for thiamin and 90 per cent for riboflavin for all methods of cooking. A wide variation in the thiamin and riboflavin contents of different pork carcasses was also reported.

Schweigert, McIntire, and Elvehjem (1943), in a study of fresh and cured hams, reported the average retention for meat alone, after roasting, to be 58 per cent for thiamin and 74 per cent for riboflavin. The average total retention in the meat plus drippings was, after roasting, 70 per cent for thiamin and 84 per cent for riboflavin; after pan broiling, 92 per cent for thiamin and 86 per cent for riboflavin. Considerable variation was noted in the thiamin content of different sections of the ham while the riboflavin values were more constant.

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[2] The authors wish to acknowledge the assistance of Miss Mary F. Gyles in the analytical work, and of Dr. Ralph Comstock in the statistical analysis of the data.

Cheldelein and Williams (1943) have recently published riboflavin values for bacon, ham, and loin chops before and after frying. The values for the raw edible portion were bacon 3.1, ham 2.6, and loin chops 2.3 micrograms per gram. The total losses after frying were, respectively, 9, 19, and 26 per cent (total loss did not include vitamin loss in cooking liquors).

Lane, Johnson, and Williams (1942) have reported thiamin values for raw edible portions of pork products as follows: ham 11.81, loin chops 14.84, and shoulder 10.51 micrograms per gram. Frying the ham incurred a thermal loss of thiamin of 47 per cent, baked loin chops lost 70 per cent, and roast shoulder lost 55 per cent of the thiamin.

The work here reported adds to existing information regarding the distribution of riboflavin and thiamin in pork loin muscles and includes studies on the effect of roasting to an internal temperature of 85°C. (185°F.) on riboflavin and thiamin losses.

EXPERIMENTAL PROCEDURE

Riboflavin: The animals used for this study were butcher hogs which were fed various fattening rations and slaughtered at a weight of approximately 225 pounds. The chief differences among the fattening rations were in the varying amounts of soybeans, soybean oil meal, and soybean oil that were fed. It should be emphasized that the rations were not designed to vary in riboflavin content. A total of 10 left loins were taken for riboflavin analysis. Roasts from these loins weighing approximately 750 grams and consisting of the first, second, and third lumbar vertebrae were cooked to an internal temperature of 85°C. The thermometer was inserted near the center of the rib eye (longissimus dorsi) muscle. After cooking, samples were taken from the rib eye and tenderloin (psoas major) muscles for determination of the moisture, ether extract, and riboflavin content. The same analyses were made on the uncooked muscles in the fourth lumbar region. The riboflavin determinations were made according to the fluorometric method described by Peterson, Brady, and Shaw (1943).

Thiamin: For this work, left loins from eight butcher hogs weighing approximately 225 pounds at the time of slaughter were used. Five of the hogs were fattened on a ration consisting chiefly of ground wheat while on soybean pasture. Three hogs were carried on pasture up to 100 pounds and then finished on a standard corn-fattening ration to which 10 per cent soybean oil was added. It should be noted that no attempt was made to influence the thiamin intake. The pork chops were one vertebrae length in thickness. An effort was made in the experiment to ascertain the magnitude of muscle-to-muscle variation as well as to find out whether different sections of the same muscle had identical thiamin contents. In the case of the uncooked meat, moisture, ether extract, and thiamin determinations were made on the muscles from one-half of the chops from the 10th and 11th thoracic and third and fourth lumbar regions. The remaining chops from the thoracic and lumbar regions were cooked to an internal temperature of 85°C. and analyzed. The rib eye (longissimus dorsi) muscle was analyzed in the thoracic region while in the lumbar region the tenderloin (psoas major) and rib eye muscles

were both analyzed. Thiamin was determined by a fluorometric procedure which will be described in detail in another publication. Briefly stated, the extraction and incubation of extracts with clarase are the same as for the riboflavin procedure except that samples are heated in a water bath for one hour instead of being autoclaved. Owing to the remarkable clarity of the meat extracts, the adsorption step used by Conner and Straub (1941) is omitted. Appropriate blanks and the increment owing to a known amount of thiamin are determined for each sample.

Analysis of variance and the "t" test were used for testing the significance of differences.

RESULTS

The riboflavin contents of the longissimus dorsi (rib eye) and psoas major (tenderloin) muscles in the lumbar region of the pork loin (Table 1) show that the wide differences in values for the two muscles are consistent for both the cooked and uncooked meat. These values are of the same magnitude reported by Peterson *et al.* (1943). The larger values of the cooked meat (Table 1) are due to the reduced moisture content and may be somewhat misleading unless it is remembered that they are on strictly a gross-weight basis. The term "gross" weight is used throughout to replace the term "fresh" weight, since the latter term might be confusing, particularly when referring to the cooked samples.

TABLE 1

Riboflavin Content of Roasts From 10 Pork Loins[1]

Muscle	Uncooked	Cooked
Longissimus dorsi	1.21 ± .09	1.40 ± .13
Psoas major	2.75 ± .20	2.88 ± .27

[1] Micrograms of riboflavin per gram of tissue, fresh-weight basis.

TABLE 2

Riboflavin Content of Pork Loin Roasts[1]

Muscle	Uncooked	Cooked	Loss
			pct.
Longissimus dorsi[2]	5.18 ± .38	4.16 ± .35	19.7
Psoas major	12.58 ± .88	10.24 ± 1.18	18.6

[1] Micrograms of riboflavin per gram of tissue (dry, fat-free basis). [2] Probability is 0.07 that the difference between the cooked and uncooked is due to chance.

The values are calculated to a dry, fat-free basis (Table 2); it will be noted that the riboflavin content of the muscles, when cooked to an internal temperature of 85°C., was uniformly lower than on the uncooked muscles. It will also be noted that on a dry, fat-free basis the cooked muscles contained approximately 80 per cent as much riboflavin per gram of lean meat as the uncooked. The apparent contradiction in the effect of cooking as shown (Tables 1 and 2) emphasizes the importance of considering vitamin values on both the gross-weight and dry, fat-free bases. Unless consideration is given this point, the values may be misinterpreted.

Average moisture and ether-extract content of the uncooked and cooked lean muscle samples are given (Table 3). Of particular interest

is the rather marked increase in the fat content of the tenderloin muscle with cooking. The increase in fat content is explained on the basis of the external fat from the top of the roast dripping down and accumulating in the tenderloin muscle. This table clearly indicates the need for calculating the vitamin values on a dry, fat-free basis in order to obtain a true picture of the losses incurred in cooking.

TABLE 3

Moisture and Ether Extract of Pork Roasts

Treatment	Longissimus dorsi			Psoas major		
	Moisture	Fat	Dry, fat-free residue	Moisture	Fat	Dry, fat-free residue
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Uncooked	72.7	3.5	23.8	75.6	2.4	22.0
Cooked	61.9	4.6	33.5	62.5	8.7	28.8

The thiamin content of the longissimus dorsi muscle in the thoracic and lumbar regions is given (Table 4); the values listed for the psoas major are for the lumbar region only since this muscle is not found in the thoracic portion of the loin. It will be noted that the values on the cooked and uncooked muscles are very similar. Here again the values may be misleading if we do not take into consideration the reduced moisture content of the cooked samples. In this table the values are listed strictly on a gross-weight basis.

TABLE 4

Thiamin Content of Chops From Eight Pork Loins[1]

Muscle	Uncooked	Cooked
Longissimus dorsi:		
Thoracic region	13.9	13.5
Lumbar region	15.1	15.9
Psoas major	14.8	13.7

[1] Micrograms of thiamin per gram of fresh lean tissue.

When the values given in Table 4 are calculated to a dry, fat-free basis (Table 5) it is noted that the thiamin values in the lumbar region

TABLE 5

Thiamin Content of Pork Loin Chops[1]

Muscle	Uncooked	Cooked	Loss
Longissimus dorsi:[2,3]			*pct.*
Thoracic region	57.8	42.2	26.6
Lumbar region	63.9	51.5	19.4
Psoas major[2]	61.8	43.2	30.1

[1] Micrograms of thiamin per gram of lean tissue (dry fat-free basis). [2] Losses in thiamin content on cooking highly significant ($P < 0.01$). [3] Thiamin content of thoracic and lumbar regions significantly different ($P < 0.05$).

of the longissimus dorsi are higher than those listed for the thoracic region. While this difference is not very large, it was statistically significant on analysis of both the cooked and uncooked samples. To the authors'

knowledge, this variation in thiamin content in different portions of the same muscle has not previously been demonstrated. It will be noted that on the dry, fat-free basis the cooked muscles contained from 20 to 30 per cent less thiamin than the uncooked. The losses were highly significant in all cases.

TABLE 6

Moisture and Ether Extract of Pork Chops

Muscle	Uncooked			Cooked		
	Moisture	Fat	Dry, fat-free residue	Moisture	Fat	Dry, fat-free residue
Longissimus dorsi:	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Thoracic region	70.4	5.7	23.9	58.2	9.8	32.0
Lumbar region	67.1	9.3	23.6	52.6	17.0	30.4
Psoas major	72.2	3.9	23.9	57.7	10.1	32.2

Average moisture and ether-extract content of the uncooked and cooked lean muscle samples are given (Table 6). The marked differences in the fat content between the two regions of the longissimus dorsi emphasize the need for considering the thiamin content on both the fresh and dry, fat-free bases. Consideration should be given the reduced moisture content of the cooked meat.

DISCUSSION

Analyses reported in this paper as well as in other unpublished work from this station have shown that marked differences may occur not only between various muscles within the same cut but differences may also be found within different sections of the same muscle. In addition to these variables, it should be pointed out that samples taken from carcasses of hogs fed identical rations vary widely in their vitamin content. Identical muscles from carcasses used in this study have varied more than 50 per cent in thiamin content. This raises two important points in regard to good sampling procedures: (1) since the animal-to-animal variation in values on the same muscle is large, values must be based on more than two or three animals to be very accurate; (2) since muscles vary in their vitamin contents, the content of a cut can be obtained only if the sample is composed of the separate muscles in the same proportion as they appear in the cut. The same concept applies to different sections of the same muscle. Results of experiments conducted at this station demonstrate the inadequacy of sampling cuts of meat without regard to some specific area which is representative of the whole cut. Probably the most satisfactory method would be to grind all of the meat in the cut and mix thoroughly enough so that analysis of aliquot samples reflects the true value of that cut.

SUMMARY

The content of riboflavin and thiamin in pork loin muscles was determined before and after cooking.

The riboflavin content of the tenderloin (psoas major) muscle was twice that of the rib eye (longissimus dorsi) muscle.

The riboflavin content of the cooked loin muscles was found to be approximately 80 per cent of that found in the uncooked when calculated to a dry, fat-free basis.

The thiamin contents of the rib eye muscle in the lumbar and thoracic regions were significantly different, the former being the higher.

The thiamin content of the cooked loin muscles was 70 to 80 per cent of that found in the uncooked when calculated to a dry, fat-free basis.

The need for standardized sampling techniques in studies of the riboflavin and thiamin contents of pork products is indicated in view of the significant differences in the vitamin content of different muscles as well as in different sections of the same muscle.

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RIBOFLAVIN CONTENT OF BEEF[1, 2]

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For the past several years the North Carolina Agricultural Experiment Station has co-operated with the Bureau of Animal Industry, United States Department of Agriculture, in a study of beef production with small grains and lespedeza—Annual Reports (1940). Since meat from cattle on this project was available for study, and since the riboflavin content of the various feeds had been determined, it seemed desirable to make additional studies on the riboflavin content of the meat produced. No data is available concerning the effect of varying intakes of riboflavin on the content of the beef. It seems generally agreed, however, that since the bovine possesses the ability to synthesize its own riboflavin, there should be no difference in the meat as reflected by various rates of intake. It is also true that there is a lack of information on the riboflavin content of beef. The few values which are reported are on only a limited number of cuts and organs. It should be pointed out that the values reported in the literature are for various cuts of beef. These cuts, however, consist of separate muscles which may vary rather widely in the riboflavin content. Work at this station has shown marked differences in the riboflavin content of various pork muscles as well as in the same muscle from different carcassses. It seemed desirable, therefore, to obtain information on the riboflavin content of the various beef muscles and organs and to study, in addition, how these values might be affected by feeding history and carcass grade.

EXPERIMENTAL PROCEDURES

Animals used for this study consisted of 12 grade Hereford yearling steers and spayed heifers. The calves were dropped in the spring and ran with their dams until they were about eight months of age. They were then weaned and carried through their first winter, in so far as possible, on winter pasture. When this was not available, the calves were full-fed lespedeza hay and two pounds of coarsely ground barley per head daily in dry lot. Summer pasture consisted principally of lespedeza. On reaching an average weight of approximately 800 pounds the yearlings grading medium or better were divided as uniformly as possible with respect to age, type, condition, and weight into three lots. Each lot was then fed as follows:

Lot 1—full-fed No. 2 (feed) barley;
Lot 2—66⅔ per cent as much grain as Lot 1;
Lot 3—33⅓ per cent as much grain as Lot 1.

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No. 2 lespedeza hay was fed *ad lib.* to all of the cattle. In addition, soybean oil meal was fed during the latter part of the feeding period to balance the rations. Four replicates, or a total of 12 cattle, were used in the riboflavin study. It was the intent to slaughter each group of three when the heaviest individual reached 1,000 pounds. This, however, was not possible in all cases. After slaughter, carcass grades and measurements were taken. Seven days later the cuts and muscles were removed from the carcass for riboflavin determinations. A total of 20 different muscles were removed from the sixth and seventh prime ribs, chuck, round, and short loin. The names of the individual muscles are listed elsewhere in this paper. In addition, the tongue, spleen, liver, and heart were analyzed for riboflavin content (Table 4).

The riboflavin determinations were made according to the fluorometric method described by Peterson, Brady, and Shaw (1943). This method is essentially that of Conner and Straub (1941) except that the adsorption and permanganate steps are omitted.

As regards the statistical analysis the significance of differences was based on analysis of variance computed from data on each cut.

RESULTS

Essential data regarding the characteristics of the live animals and carcasses as well as the feeding data are shown (Table 1).

TABLE 1

Feed-Lot and Carcass Characteristics on Three Lots of Cattle

Characteristics	Lot 1 (Full-fed grain)	Lot 2 (66⅔% of Lot 1)	Lot 3 (33⅓% of Lot 1)
Average feeder grade [1]	12.5	13.0	15.5
Average slaughter grade [2]	10.5	11.5	18.0
Average carcass grade [2]	10.5	12.0	17.5
Average dressing per cent (cold)	59.3	58.0	54.7
Average weight of feeder (pounds)	640.0	660.0	625.0
Average final weight (pounds)	942.0	907.0	770.0
Average daily gain (pounds)	1.47	1.21	0.71
Average age at slaughter (days)	766.0	772.0	761.0
Total feed consumed per cwt. gain (pounds) [3]:			
Ground barley	680.0	572.0	486.0
Soybean oil meal	29.1	24.8	21.1
Lespedeza hay	524.0	939.0	1811.0
Riboflavin intake (grams):			
For total feeding period	7.25	8.32	8.18
Average daily	0.035	0.041	0.040
Per cwt. gain	2.42	3.47	5.86

[1] Scores range from 1 to 36 with the smaller number representing the highest grade. [2] Scores range from 1 to 42 with the smaller number representing the highest grade. [3] The average riboflavin contents of the ground barley, soybean oil meal, and lespedeza hay were 2.76, 3.60, and 5.04 micrograms per gram, respectively.

That there are significant differences in the riboflavin content of the various muscles is indicated (Table 2); the trapezius thoracalis, for example, has 46 per cent more riboflavin than the longissimus dorsi in the prime rib. Similar differences may be observed between muscles of

TABLE 2

Riboflavin Content of Fresh Beef Muscle[1]

Wholesale cut	Muscle	Mean value	Least sig. dif.[2]
Prime rib		1.72	.23
	Longissimus dorsi	1.44	
	Latissimus dorsi	1.51	
	Rhomboideus thoracalis	1.81	
	Trapezius thoracalis	2.10	
Chuck		1.78	.28
	Triceps (lateral head)	1.92	
	Triceps (long head)	1.71	
	Biceps (brachii)	1.91	
	Coraco-brachiolis	1.71	
	Posterior deep pectoral	1.73	
	Deltoideus	2.02	
	Serratus thoracic	1.45	
Round		1.63	.29
	Vastus intermedius	1.82	
	Sartorius	1.69	
	Biceps femoris	1.78	
	Semitendinosus	1.20	
	Semimembranosus	1.63	
Short loin		1.84	.23
	Longissimus dorsi	1.59	
	Psoas major	2.09	
	Serratus dorsalis	1.30	
	Longissimus costarum	1.93	
	Transversus abdomenes	2.28	

[1] Micrograms of riboflavin per gram of lean tissue. [2] Between muscles within cuts at the five-per cent level.

TABLE 3

Riboflavin Content of Meat From Three Levels of Grain Intake[1]

Cut of meat	Lot 1	Lot 2	Lot 3
Chuck	1.87	1.71	1.75
Prime rib	1.73	1.73	1.70
Short loin	1.80	1.85	1.86
Round	1.61	1.55	1.72
Average	1.75	1.71	1.76

[1] Micrograms of riboflavin per gram of lean tissue.

TABLE 4

Average Riboflavin Content of Beef Organs[1]

Tongue	3.07 ± 0.17
Spleen	3.87 ± 0.07
Liver	27.21 ± 1.13
Heart	10.66 ± 0.11

[1] The riboflavin values listed in Table 4 were obtained from the organs of 12 cattle. Values are given in micrograms of riboflavin per gram of fresh tissue.

the various other cuts. The riboflavin content of the muscles from the round was significantly lower than from the muscles of the prime rib and short loin.

No significant difference was found in the riboflavin content between the meat from any of the three lots (Table 3). It should be pointed out that the lean meat of the No. 1 lot contained considerably more fat than the No. 3 lot.

DISCUSSION

The data presented raise a number of questions which cannot be answered from the information available at this stage of the study. Of particular interest is the rather wide variation in the riboflavin content of the various beef muscles and yet the marked lack of variation from cut to cut. One can conclude that for the animals studied the riboflavin content of beef muscle does not vary to any appreciable extent from cut to cut nor is it affected by the degree of fatness. Certainly there seems to be no indication of a correlation between certain palatability factors and the riboflavin content. It is evident that the riboflavin intake per hundredweight of gain had no effect on the amount stored. Since the daily average intake was very nearly the same for all three lots, no relationship could be found here.

From the data presented it may be suggested that in studies of the riboflavin content of meat, determinations should be made either on particular muscles within a given cut, or all of the meat in a cut should be ground finely and mixed thoroughly enough so that analyses of aliquot samples reflect the true riboflavin value of the cut.

SUMMARY

The riboflavin content is given for 20 beef muscles and four organs from 12 carcasses of different grade and feeding history.

Significant differences were found between the riboflavin content of various muscles within four different cuts of beef. In no case, however, were as wide differences shown between beef muscles as had previously been demonstrated in pork muscles.

The average riboflavin content of the muscles which constitute beef cuts differed significantly in some cases; not at all in others.

No relationship was found between the rate of riboflavin intake per hundredweight of gain and the amount deposited in the lean.

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THERMOPHILIC BACTERIA IN FOODS AND IN VARIOUS INGREDIENTS ENTERING INTO THE MANUFACTURE OF FOODS

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That thermophilic bacteria are widely distributed in nature is now a well-established fact. It is further known that certain food ingredients, such as sugar and starch, have been in the past a major source of the thermophiles causing spoilage in many types of canned goods. Special grades of starch and sugar are now being produced for the canning industry in which the thermophiles have either been eliminated or reduced to negligible amounts. Many minor ingredients other than starch and sugar are added to processed foods, including water, fillers, emulsifiers, spices, flavorings and food colors. These materials are also a potential source of thermophiles contaminating processed foods. This paper records the analysis of materials of this type for the presence of organisms which develop in media incubated at 55°C.(131°F.). The water samples consisted of specimens from private wells and dairy water supplies that have been sent into this laboratory during the year 1943 for examination for fitness for human consumption. The other ingredients were obtained direct from the manufacturers and in each instance they were supposed to be first-grade products.

PROCEDURES

The thermophilic aerobes were counted on plates poured with tryptone (1 per cent), dextrose (0.5 per cent), and agar (1.5 per cent). Approximate counts of thermophilic *Clostridia* were obtained by making dilutions of the materials examined in corn-meal-liver mash by McClung and McCoy (1934), and grass medium, Castell (1941). With the water samples, dilutions were never made; each test represents a direct transfer of one milliliter of water into the enrichment media. All other materials were prepared in aqueous dilutions from 1 to 1 in 1,000,000, and one-ml. transfers were made from each of these dilutions into the enrichment media. These were incubated at 55°C. from seven to 14 days. At the end of this period, cultures in which gas had been produced were steamed for six minutes to kill the vegetative cells and reinoculated into fresh enrichment media. They were then reincubated for at least 14 days at 55°C. The rapid evolution of gas and the presence of plectridial or clostridial forms during this second incubation period was considered indicative of the presence of anaerobes. In a few instances spores were not observed under the microscope, but they were considered to be present when the cultures remained alive through a series of steamings followed by transfers into fresh enrichment media.

Where anaerobic growth occurred in one enrichment medium and not in the other, or in higher dilutions in one medium, the positive or higher

results were recorded. Previous tests by Castell (1941) have shown that the comparative value of these two enrichment media for initiating growth from small inocula of pure cultures varies greatly with the species used. For this reason it was considered that the highest counts in either medium gave a more accurate picture than one medium by itself or the average of both. All tests were made in duplicate.

WATER

Of 281 samples tested, it was found that 69 (24.5 per cent) contained thermophilic, sporeforming anaerobes and that 99 (35.2 per cent) contained thermophilic aerobes. Only six samples gave counts of more than 100 aerobes per milliliter; the average of the remaining 93 samples containing these organisms was 3.5 colonies per milliliter. In some cases the rapid growth of spreaders made counting difficult, but these were too few to cause any significant error in the results. Two thermophilic molds (both Mucors) and 23 actinomycetes were also observed on the plates at 55°C.

Of this first group of 281 water samples, 229 were also tested for pollution as indicated by the presence of *Escherichia coli*; 64 samples (27.9 per cent) were positive. Of these 64 polluted samples, 23 contained no thermophiles; 22 contained only thermophilic aerobes; eight contained only thermophilic sporeforming anaerobes; and 22 contained both types. A group of 169 water samples were tested for both thermophilic and mesophilic *Clostridia* by incubating the enrichment media at 55 and 37°C. (131 and 98.6°F.). The object was to observe the relative frequency of the two groups in water, and more especially to determine whether or not there was any association between the presence of the two groups. The results showed that 131 (77.5 per cent) contained *Clostridia* which developed at 37°C., and 41 (24.2 per cent) which grew at 55°C. There was only one water sample from which *Clostridia* grew at 55°C. and not at 37°C. Repeated microscopical examinations showed that 83 per cent of the samples producing anaerobic sporeformers at 37°C. contained some typical granulose-bearing clostridial types, suggestive of the butyric acid-forming group.

FOOD COLORINGS

Twenty-eight samples were analyzed. Five were liquid, cheese and butter colorings; these contained no thermophiles. A thick, syrupy, amber coloring had 57 thermophilic aerobes and between 10 and 100 thermophilic anaerobes per gram. Twenty-two samples were in powder form; of these, 12 contained no thermophiles; seven contained no anaerobes and from one to 150 aerobes per gram; two contained between 10 and 100 anaerobes and between 10 and 200 aerobes; and one sample had between 10,000 and 100,000 anaerobes and 65,000 aerobes per gram. This would indicate that only one sample, a powdered orange coloring, had significant amounts of thermophiles present.

FOOD FLAVORINGS

Only eight samples were tested. Seven were liquid and these contained no thermophiles. A powdered chocolate flavoring contained between 1,000 and 10,000 anaerobes and 3,200 aerobes per gram.

SPICES

Thermophilic counts for 20 samples of spices (Table 1) show that as a group these materials are much more seriously contaminated with thermophiles than either the food colorings or food flavorings. In the lower dilutions it was observed that a few of the spices had an inhibiting action on the growth of the bacteria. This was particularly noticeable with cloves in the anaerobic cultures and cassia in the aerobic thermophiles. However, the dilutions in which this inhibiting action was observed were too low for it to have any practical application in checking the growth of thermophilic anaerobes in foods.

TABLE 1

Thermophiles in Spices

Material	Aerobes [1]	Sporeforming anaerobes [2]
Ground white pepper	7,800,000	1,000,000
Ground ginger	6,600,000	1,000
Ground alleppy turmeric	3,800,000	1,000
Ground Portugese paprika	800,000	1,000,000
Pork sausage seasoning	700,000	10
Ground black pepper	600,000	1,000
Ground savory	320,000	1,000
Ground coriander	300,000	10
Ground sage	40,000	100
Ground thyme	32,000	1,000
Ground cloves	16,000	100,000
Ground allspice	5,000	10
Cayenne pepper	1,000	10
Ground nutmeg	350	10,000
Sassafras bark	130	10
Frankfurt seasoning	30	1
Pork sausage seasoning	10	1
Coumarin	7	0
Vanillin powder	0	0
Ground Batavia cassia	?[3]	1,000,000

[1] Plate counts per gram. [2] Dilution counts in enrichment media. [3] Counting was made difficult because of the inhibiting action of the spice on the development of colonies.

CEREAL PRODUCTS, FILLERS, ETC.

The counts for this group are given (Table 2); the unprocessed corn flour, the untreated soya flour, and the low-grade wheat flour were added for comparison with the first-grade and processed products.

Most of the aerobic thermophiles with this group were sporeformers. An incidental experiment with the untreated soya flour gives some idea of the heat resistance of the organisms in one of these highly contaminated products. A mixture was made of the soya flour (five per cent), sterile calcium carbonate (two per cent), sterile agar (two per cent), and sterile water. This was prepared in 156 20-ml. lots in small, sterile, screw-capped bottles. They were all placed in flowing steam and six were removed after 5, 10, 15, and 20 minutes, and then every 10 minutes for the first hour and at half-hour intervals for 10 hours. As soon as the bottles were taken from the steamer, three were placed at 37°C. and three at 55°C. The flour itself acted as an enrichment medium. In every single bottle

TABLE 2

Thermophiles in Cereal Products, Emulsifiers, Fillers, Etc.

Material	Aerobes [1]	Sporeforming anaerobes [2]
Unprocessed corn flour	12,500,000	10
Food stabilizer	4,800,000	1,000,000
Food stabilizer	3,000,000	100,000
Untreated soya flour	2,100,000	100,000
Starch	37,000	100
Baker's starch	21,500	10
Starch	14,500	100
Low-grade wheat flour	13,000	100
Ground starch	7,000	10
Potato starch	7,000	0
Irish moss	6,000	10
Sodium alginate	5,200	10
Tragacanth	4,000	10
Processed corn flour	1,000	10
Acacia	800	10
Soya flour	630	10
Tapioca starch	480	10
"Wheatlets"	450	10
Cornstarch	350	10
Acacia	180	100
Wheat flour	80	10
Wheat flour	80	0
Irish moss	80	10
Cornstarch	25	10
Canning starch	0	0
Emulsifier	0	0
Potato flour	0	0
Powdered sugar	0	1
Cane sugar	0	10
Cane sugar	0	0

[1] Plate counts per gram. [2] Dilution counts in enrichment media.

TABLE 3

Thermophiles in Dairy Products and Miscellaneous Materials

Material	Aerobes [1]	Sporeforming anaerobes [2]
Spray whole milk powder	8,000,000	10
Powdered skim milk	210,000	100,000
Cocoa	11,000	100
Powdered milk	3,000	10
Cheddar cheese	2,700	0
Cheddar cheese	2,200	0
Whole milk powder	2,000	10
Powdered gravy	1,060	1
Whole milk powder	780	10
Partly skim-milk powder	480	0
Annato seed	265	100
Skim-milk powder	160	10
Dried-egg powder	0	0
Salad oil	0	0

[1] Plate counts per gram. [2] Dilution counts in enrichment media.

the development of surface growth or a heavy pellicle showed the presence of both mesophilic and thermophilic aerobic sporeformers. Tests for *Clostridia* showed that both mesophilic and thermophilic types were present in most of the bottles that had been heated up to 50 minutes. Mesophilic *Clostridia* were also obtained from two bottles that had been steamed for four and four and one-half hours, respectively.

ADDITIONAL FOODS AND DAIRY PRODUCTS

A number of materials not included in the preceding categories, but which might also find their way into canned foods, were also tested (Table 3).

SUMMARY AND CONCLUSIONS

Four hundred and fifty water samples were tested for the presence of thermophilic bacteria. Aerobic types, usually in very small numbers, were found present in approximately 35 per cent; sporeforming anaerobes were present in approximately 25 per cent.

In the analysis of 169 samples it was found that mesophilic *Clostridia* were more than three times as prevalent as the thermophilic *Clostridia*. In only one instance did a water sample contain thermophilic *Clostridia* without also containing *Clostridia* which developed at 37°C.

Aerobic thermophiles were present in 68.6 per cent of 64 polluted water samples; 46 per cent of this same group contained thermophilic *Clostridia*; 22 polluted samples (34.3 per cent) contained no thermophiles.

One hundred food products or ingredients used in food products were also examined for thermophiles. Spices, unprocessed cereal products, food stabilizers, and certain of the powdered dairy products contained relatively large numbers of both aerobic and anaerobic organisms. They were present in very small numbers or entirely absent from most food colorings, flavorings and specially processed sugars, and cereal products.

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VITAMIN CONTENT OF SOME TROPICAL FRUITS, THEIR JUICES AND NECTARS

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The present report deals with the determination of carotene, thiamin, riboflavin, and ascorbic acid in certain tropical and subtropical fruits. These studies include guavas, papayas, and mangoes obtained from the Florida Subtropical Experiment Station.[1] Lemons, oranges, and grapefruit were purchased on the local market. Canned orange juice, grapefruit juice, vegetable juice cocktails, tomato juice, and apricot nectar were also purchased on the open market. Nectars of guava, mango, tamarind, banana, quenepa, papaya, and soursap and pineapple juice were prepared by the Division of Investigation and Development of the Department of Agriculture of Puerto Rico or by Puerto Rican commercial concerns.

EXPERIMENTAL PROCEDURE

Chemical methods were employed for vitamin assay. Carotene was determined by extraction of the pigments with petroleum ether after saponification with 10-per cent alcoholic NaOH, chromatographic separation of pigments in the petroleum ether extract by means of a dicalcium phosphate column, and measuring the carotene against pure β carotene as a reference standard in a Cenco-Sheard spectrophotelometer.

Thiamin was determined by the thiochrome method of Hennessy and Cerecedo (1939), using the Pfaltz and Bauer fluorometer. Riboflavin was also measured fluorometrically according to the method of Conner and Straub (1941). Ascorbic acid was estimated by direct titration with 2,6-dichlorobenzeneoneindophenol as originated by Tillmans and modified by Bessey and King (1933), except that extraction was performed in a Waring blender with trichloroacetic and metaphosphoric acids.

A summary of results[2] obtained on four tropical fruits and pineapple juice for carotene, thiamin, riboflavin, and ascorbic acid is given (Table 1); the data obtained on the ascorbic acid content of nectars and juices is shown (Table 2).

DISCUSSION

Owing to war conditions it was impossible to study varieties, stages of ripeness, etc., as originally planned. The mangoes were all of the Haden

[1] We wish to acknowledge the helpful co-operation of Dr. G. D. Ruehle who furnished some of the subtropical fruits used in these studies.

[2] Since the above data were submitted for publication the following subtropical fruits were received from Dr. Philip J. Westgate of the Subtropical Experiment Station, Homestead, Florida, and were found to contain the following amounts of ascorbic acid: (1) carambola, 35.3 milligrams per 100 grams fresh fruit; (2) carissa, 6.48 milligrams per 100 grams fresh fruit; (3) loquats (overripe), 3.96 milligrams per 100 grams fresh fruit.

TABLE 1

Vitamin Content of Some Tropical Fruits

Fruits	Condition of fruit	Carotene	Thiamin	Riboflavin	Ascorbic acid
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Papaya No. 1	Fairly ripe	.....	.....	.....	43
Papaya No. 2	Fairly ripe	.903	.042	.050	29
Papaya No. 3	Fairly ripe	.790	.042	.038	32
Papaya No. 4	Ripe	.990	.045	.038	63
Papaya No. 5	Somewhat green	.713	.039	.052	40
Guava No. 1	Ripe and hard	.307	.042	.027	246
Guava No. 2	Overripe, soft	.430	.039	.032	190
Guava No. 3	Ripe and hard	.025	.045	.017	296
Guava No. 4	Overripe, soft	.014	.042	.027	196
Orange No. 1	Ripe	.109	.117	.028	35
Orange No. 2	Ripe	.118	.105	.034	40
Orange No. 3	Ripe	.127	.117	.024	44
Mango No. 1	Ripe	.506	.060	.073	17
Mango No. 2	Ripe	.505	.063	.037	15
Mango No. 3	Ripe	.527	.057	.050	17
Pineapple juice No. 1	From cull fruits	.023	.051	.017	21
Pineapple juice No. 2	From cull fruits	.026	.051	.023	21

TABLE 2

Ascorbic Acid Content of Various Nectars and Juices as Determined by Dye-Titration Method

Sample of material	Description	Ascorbic acid (vitamin C)
		mg./ 100 ml.
Pineapple juice	Canned from cull pineapples in Puerto Rico (1941)	22
Papaya nectar	Canned (Puerto Rico, 1941)	16
Guava nectar	Canned (Puerto Rico, 1941)	51
Tamarind nectar	Canned (Puerto Rico, 1941)	5
Mango nectar	Canned (Puerto Rico, 1941)	20
Banana nectar	Canned (Puerto Rico, 1941)	3
Soursap nectar	Canned (Puerto Rico, 1941)	11
Quenepa nectar	Canned (Puerto Rico, 1941)	2
Grapefruit juice	Canned (local market)	44
Orange juice	Canned (local market)	40
Tomato juice	Canned (local market)	22
Vegetable juice cocktail No. 1	Canned (local market)	7
Vegetable juice cocktail No. 2	Canned (local market)	10
Apricot nectar	Canned (local market)	3
Lemon juice	Fresh (local fruit market)	36
Orange juice	Fresh (local fruit market)	53
Grapefruit juice	Fresh (local fruit market)	39

variety and were at practically the same stage of ripeness. Consequently, no marked differences are noted in vitamin content. The pineapple juices were known to be made from culls which had ripened in the Puerto Rican warehouse and are probably not comparable with high-grade pineapple juice.

The carotene content of papayas tends to increase with ripening. There was no uniformity in the carotene content of guavas, of which there were two varieties, and regarding which no information was available. One variety of guava is represented by Samples 1 and 2 and the other by Samples 3 and 4 (Table 1). Apparently, the ascorbic acid content of guavas tends to decrease as they become overripe. It would appear that this fruit is an excellent source of ascorbic acid, being superior to oranges in this respect.

SUMMARY

The carotene, thiamin, riboflavin, and ascorbic acid content of papayas, guavas, mangoes, oranges, and commercially canned pineapple juice have been determined by chemical methods. The ascorbic acid content of a number of fruit juices and fruit nectars have also been studied. The limited data seem to indicate that papayas are an excellent source of carotene and a good source of ascorbic acid but are less rich in thiamin and riboflavin than the other fruits.

Guavas are excellent sources of ascorbic acid while carotene seems to be the principal factor contributed by the mango. Guava nectar compares very favorably with orange juice and lemon juice as a source of ascorbic acid.

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INFLUENCE OF CALCIUM AND MAGNESIUM UPON COMPOSITION OF BOSTON HEAD LETTUCE [1]

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Lettuce, one of our principal vegetable crops, is extensively grown practically throughout the United States by both commercial and home gardeners. In some parts of the United States it is a year-round crop and in the Massachusetts locality it is one of the principal winter greenhouse vegetable crops. Accordingly one may purchase lettuce in the retail markets practically all through the year. It is relished by nearly everyone and is extensively used as a component of salads. Consequently it is not surprising that the value of a year's lettuce crop in the United States has been estimated by the U. S. Department of Agriculture (1942) to be about $40,000,000.

For many years it has been classed as a protective food since it contains minerals and vitamins which are not present in adequate amounts in carbohydrate-rich and fat-rich foods. While meat and dairy products are also rich sources of minerals and vitamins, they are much more expensive and at the present time they can be purchased in only limited amounts. Hence, it is important to evaluate carefully the extent to which lettuce may be relied upon as a protective food. A survey of the literature, however, did not reveal as complete information concerning the mineral and vitamin content of lettuce as desired.

Commercial and practical gardeners are of the opinion that frequently the quality and yield of lettuce can be enhanced by supplementing the usual lettuce fertilizers with lime or lime and magnesium. For several years the Agronomy Department has been collecting data on this point, and it has studies in progress to determine the economic value of using lime and magnesium as supplementary fertilizers for lettuce. Accordingly it seemed desirable to determine whether the mineral and vitamin content of lettuce is significantly influenced by the use of these supplementary fertilizers.

EXPERIMENTAL PROCEDURE

A study has been made of the composition of Boston head lettuce produced on four plots of land that received different fertilizer treatments. During the years 1938 to 1942, inclusive, the four experimental plots received a home-mixed 5-8-7 fertilizer at the rate of 2,000 pounds per acre. The ingredients were sodium nitrate (16 per cent nitrogen), ammonium sulfate (20 per cent nitrogen), potassium chloride (60 per cent potassium oxide), and ammonium phosphate. These ingredients were mixed in such proportions that one-third of the nitrogen was obtained from each of the nitrogen-containing ingredients. Because of war restric-

[1] Contribution No. 509 from the Massachusetts Agricultural Experiment Station.

tions it was impossible to follow this program during the 1943 season. Accordingly a commercial 4-9-7 fertilizer was used on all four plots (A, B, C, D) at the rate of one ton per acre. For approximately 20 years these plots annually received additional magnesium or calcium as follows:

Plot A—control plot;
Plot B—magnesium sulfate at the rate of 150 lb. an acre;
Plot C—magnesium sulfate at the rate of 150 lb. and limestone at the rate of 1,000 lb. an acre;
Plot D—limestone at the rate of 1,000 lb. an acre.

The mineral and vitamin values of the lettuce grown on these four experimental plots were judged by the amount of carotene, riboflavin, calcium, iron, magnesium, and phosphorus that it contained.

ASSAY PROCEDURES

The Boston head lettuce used in this study was produced from seed that was sown in the greenhouse April first. On May first the plants were transferred to the four experimental plots described above. Two months later, when the lettuce had grown to the market or harvesting stage, typical mature heads were selected for assay. At this time good, firm, well-developed heads were picked from each of the four plots on four different dates, i.e., June 30 and July 5, 8, and 12. The lettuce was promptly taken to the laboratory and all the outer leaves were removed and discarded. An attempt was made to remove as many leaves as would have been discarded by the grower, wholesaler, retail grocer, and the housewife. The leaves that were discarded represented quite a portion of the total volume of the lettuce heads as they were taken from the field but it was desired to retain for assay only that portion of the lettuce heads which would normally be used for food. After the outer leaves were removed, the lettuce heads were of a light green color similar to the usual market head lettuce. The remaining leaves were separated from each other and immediately cut into very fine particles with shears. The finely cut material was then separated into aliquots for the various assays, the appropriate liquids were added, and the lettuce was further disintegrated with a Waring blender. In general the samples for the assay procedures were prepared and weighed within one hour from the time the lettuce was pulled from the ground.

The water, carotene, riboflavin, calcium, iron, magnesium, and phosphorus contents of the lettuce were determined by recognized assay procedures. The carotene was determined by a rapid chromatographic method described by Wall and Kelley (1943), and the fluorometric method described in detail by Holmes (1944) was used for riboflavin. Calcium was determined by the oxalate method, iron by the colorimetric method, magnesium by the magnesium pyrophosphate method, and phosphorus by the volumetric method, all of which are official methods of the Association of Official Agricultural Chemists (1940).

RESULTS

The results of the assays of lettuce selected from the four experimental plots on four different dates (Table 1) reveal that the water content of

lettuce from the different plots did not vary greatly and averaged approximately 95 per cent. The values reported (Table 1) for carotene, riboflavin, calcium, iron, magnesium, and phosphorus are computed on the fresh basis. For the sake of convenience, these data will be discussed under separate headings.

TABLE 1

Influence of Calcium and Magnesium Upon Composition of Boston Head Lettuce[1]

Source	Water	Carotene	Riboflavin	Calcium	Iron	Magnesium	Phosphorus
	pct.						
Plot A	94.7	2.7	.10	29.6	2.6	15.6	23.8
B	95.6	3.4	.09	24.6	1.9	17.4	21.2
C	96.2	2.8	.08	22.1	1.5	14.6	21.7
D	96.4	5.0	.08	25.9	2.0	9.4	20.8
A	94.0	3.0	.06	27.3	2.4	15.3	26.6
B	95.0	2.4	.06	18.5	2.0	14.7	22.9
C	95.4	3.4	.07	23.9	1.5	12.4	22.9
D	96.1	1.0	.07	20.3	1.1	7.2	19.8
A	93.6	2.6	.07	35.0	4.4	14.3	25.0
B	94.3	3.4	.07	22.0	1.8	16.4	23.3
C	95.3	1.3	.07	21.9	1.9	13.1	26.1
D	95.0	2.3	.06	26.3	1.8	10.6	26.9
A	94.7	3.2	.09	22.1	2.5	12.8	23.9
B	95.3	2.8	.10	28.1	2.2	18.0	20.7
C	96.0	2.0	.09	18.6	1.8	10.9	23.6
D	96.3	2.0	.08	17.5	1.7	8.4	22.6
Average							
A	94.3	2.9	.08	28.5	3.0	14.5	24.8
B	95.1	3.0	.07	23.3	2.0	16.6	22.0
C	95.7	2.4	.08	21.6	1.7	12.8	23.8
D	95.9	2.6	.09	22.5	1.7	8.9	22.5

[1] Given in milligrams per 100 grams, fresh basis.

Carotene: The carotene content of the lettuce varied from plot to plot on each of the different harvesting dates. However, the average values obtained for the carotene content of the lettuce were fairly uniform, being 2.9 mg. per 100 grams for Plot A, 3.0 mg. for Plot B, 2.4 mg. for Plot C, and 2.6 mg. for Plot D. It is probable that these carotene values are lower than would have been obtained if the outside green leaves, which are normally discarded during the marketing of head lettuce, had been included in the sample. Unfortunately the literature contains few reports of the carotene content of lettuce.

Olcovich and Mattill (1930) obtained 250 milligrams of carotene from 150 kilograms of lettuce. Euler, Demole, Karrer, and Walker (1930) found that the vitamin A activity of the ether extract of lettuce was proportional to the carotene content of the lettuce. Steenbock and Sell (1922) reported that green cabbage leaves are richer in the fat-soluble vitamin and contain ten times as much yellow pigment as the white leaves. Dye, Medlock, and Crist (1927) stated the outside leaves of head lettuce are far superior to the inside leaves as a source of vitamin A, and while

there is no proven identity of chlorophyll or any of its primary phases with vitamin A, there is a probability of close relationship between the two. Kramer, Boem, and Williams (1929) found the dark green outside leaves of California head lettuce contained 30 or more times as much vitamin A as equal weights of the white center leaves from the same heads.

Riboflavin: The riboflavin content of the lettuce varied from plot to plot for each of the four different dates on which it was harvested. However, the variations were not large and the average values for the different plots were in good agreement, being 0.08 mg. for Plot A, 0.07 mg. for Plot B, 0.08 mg. for Plot C, and 0.09 mg. of riboflavin per 100 grams for Plot D. Also it may be noted that these values are within the range of those found in the literature. Wilson and Roy (1938) report that lettuce grown in India contained 116 micrograms of riboflavin per 100 grams. Hodson (1940) found 0.48 microgram of riboflavin per gram for head lettuce, 0.96 for stemmy lettuce, and 0.71 for leaf lettuce. Poe and Hartman (1940) concluded from feeding Colorado head lettuce to rats that both the outer and inner leaves contained 55 to 67 Sherman units of riboflavin per 100 grams. Cheldelin and Williams (1942) found the riboflavin content of lettuce to be 27 micrograms per 100 grams. Munsell (1942) studied lettuce by the rat-growth method and concluded that it contained less than 0.25 microgram of riboflavin per gram.

Calcium: The amount of calcium found in the lettuce varied with the plot on which it was grown and with the different dates of harvesting but the time element involved in the harvesting dates is not significant. Consequently interest is centered upon the average values obtained for the different plots. These were 28.5 mg. of calcium per 100 grams of lettuce for Plot A, 23.3 mg. for Plot B, 21.6 mg. for Plot C, and 22.5 mg. for Plot D. These values are in agreement with those reported by a number of investigators.

Crist (1925) stated that Michigan lettuce contained from 0.61 to 1.62 per cent of calcium. In their assay of Arizona lettuce, McGeorge, Wharton, and Frazier (1940) obtained 0.9 per cent calcium; and Zimmerley (1930) found from 0.94 to 1.28 per cent of calcium in Virginia lettuce. Kohman (1939) obtained 0.073 per cent of calcium, and Davidson and LeClerc (1936) found from 0.33 to 0.45 per cent of calcium in lettuce, with marked variation in plants from different localities. Yeh and Adolph (1938) reported that lettuce grown in China contained 0.066 per cent of calcium. Mallon and Urey (1941) found that the calcium content of California Iceberg lettuce varied from 0.019 to 0.033 per cent for the inner leaves and from 0.089 to 0.111 per cent for the outer leaves and that the calcium content of New York Imperial lettuce varied from 0.113 to 0.196 per cent for the outer leaves and from 0.030 to 0.031 per cent for the inner leaves. In their study the outer leaves represented one-third of the entire weight of the plant. The U. S. Department of Agriculture (1938) reported that the very green lettuce leaves are richer in calcium.

In experiments with two healthy young women, Mallon, Johnson, and Darby (1933) found that the utilization of calcium of the fresh green leafy lettuce, which furnished 98 per cent of the total calcium content of the diet, was superior to that of calcium in pasteurized whole milk when

these foods were consumed on an approximately equal calcium basis. In later experiments Shields, Fairbanks, Berryman, and Mitchell (1940) found that the calcium of lettuce was 80 per cent as available as the calcium of milk. Since Outhouse, Breiter, Rutherford, Dwight, Mills, and Armstrong (1941) have reported that the calcium in milk is 24 per cent utilized by the human body, these data indicate that when one consumes lettuce as a part of a normal mixed diet nearly 20 per cent of the calcium supplied by the lettuce can be utilized by the body. Hence the body would obtain about five milligrams of calcium from 100 grams of the lettuce under discussion.

Iron: Iron varied from a minimum of 1.1 milligrams per 100 grams of lettuce for Plot D at the second harvesting to a maximum of 4.4 milligrams for Plot A at the third harvesting. The average amounts of iron found in the lettuce on the four harvesting dates were 3.0 mg. per 100 grams of lettuce for Plot A, 2.0 mg. for Plot B, and 1.7 mg. for Plots C and D. These data seem to indicate that the addition of the supplementary magnesium and calcium salts to the soil of Plots B, C, and D tended to decrease the iron content of the lettuce. However, it is interesting to compare these results with those reported by other investigators.

In a study of the iron content of foods used in a municipal hospital, Toscani and Reznikoff (1934) found the iron content of heart lettuce to be 0.50 mg. and of leaf lettuce, 1.04 mg. per 100 grams. The former value was substantiated by Chatfield and Keiles (1941), who reported that the iron content of fresh head (bleached) lettuce was 0.5 mg. per 100 grams. Remington and Shiver (1930) concluded that lettuce (dry basis) contained 2,110 p.p.m. of iron. Levine, Culp, and Anderson (1932) found lettuce contained 0.237 mg. of iron per 100 grams but the iron content varied considerably with localities. Peterson and Elvehjem (1928) reported that head lettuce contains 0.00042 per cent and leaf lettuce 0.00187 per cent of iron.

In their study of ionizable iron in foods, Shackleton and Chance (1936) found that lettuce contained 0.80 mg. of iron per 100 grams and that 63 per cent of it was ionizable. Kohler, Elvehjem, and Hart (1936) found that 21 to 28 per cent of iron in lettuce was available. Aschman, Speirs, and Maddox (1938) concluded from their studies of the availability of iron in foods that dried leaf lettuce contained 0.245 mg. of iron per gram but that only 60 per cent of the iron was ionizable. Sheets (1936-37) found in studies of experimental anemia in rats that a large proportion of the iron in unashed, green, leafy vegetables, such as lettuce, was not utilized.

A number of investigators have studied the value of lettuce as a source of iron for treating nutritional anemia. Hart, Elvehjem, Waddell, and Herrin (1927) reported that nutritional anemia can be corrected by the ash of lettuce but some additional factor affecting the utilization of iron becomes operative. Aschman, Speirs, and Maddox (1938) studied the availability of iron in various foods and found that lettuce contained sufficient available iron to produce 4.7 grams (head lettuce) and 4.0 grams (leaf lettuce) gain in hemoglobin per 100 c.c. of blood. Levine, Culp, and Anderson (1932) found that when rats with nutritional anemia were fed sufficient lettuce to supply two mg. of iron daily a regeneration of blood

was brought about in six to seven weeks, but when lettuce was supplemented with tomatoes, regeneration took place in four to five weeks owing to the copper in tomatoes.

Magnesium: The magnesium content of lettuce from the different experimental plots varied over wide limits, but at each harvesting period there was a definite relation between the magnesium contents of the lettuce from the different plots. Of three of the four harvesting dates the lettuce from Plot B, which received supplementary magnesium sulfate at the rate of 150 pounds per acre, contained more magnesium than the lettuce from Plot A, which received no magnesium sulfate. In each instance the lettuce from Plot C, which received the extra magnesium sulfate as well as lime at the rate of 1,000 pounds per acre, contained less magnesium than the lettuce from Plots A or B. In all cases the lettuce from Plot D, which received 1,000 pounds of lime per acre, contained much less magnesium than the lettuce from any of the other plots. The average values were 14.5 mg. of magnesium per 100 grams of lettuce for Plot A, 16.6 mg. for Plot B, 12.8 mg. for Plot C, and 8.9 mg. for Plot D. These data indicate that supplementary lime fertilization may reduce the magnesium content of lettuce. Unfortunately the literature consulted did not contain any data concerning the effect of supplementary magnesium and calcium fertilization upon the magnesium content of lettuce. In fact, relatively few data concerning the magnesium content of lettuce were found in the literature consulted. Davidson and LeClerc (1936) stated lettuce contained from 0.19 to 0.27 per cent of magnesium but the amount varied widely depending upon the locality in which the lettuce grew. McGeorge, Wharton, and Frazier (1940) studied the mineral content of lettuce grown at different localities in Arizona. In three localities they found that the magnesium content of lettuce (dry basis) varied from 0.32 to 0.57 per cent, from 0.07 to 0.54 per cent, and from 0.16 to 0.65 per cent. The average values for these three localities were 0.43, 0.30, and 0.38 per cent.

Phosphorus: The phosphorus content of the lettuce was similar for the four experimental plots. At three of the four harvest periods the phosphorus content of the lettuce from Plot A (control) was greater than for the lettuce from the other three plots. The average values were 24.8 mg. of phosphorus per 100 grams of lettuce for Plot A, 22.0 mg. for Plot B, 23.8 mg. for Plot C, and 22.5 mg. for Plot D. It is evident from these values that the average amount of phosphorus present in the lettuce did not vary widely. Accordingly, there was no evidence that the supplementary magnesium and calcium fertilizers materially affected the phosphorus content of lettuce, although the phosphorus content of the lettuce tended to decrease with the increase in the amount of calcium in the fertilizer. This is in agreement with the conclusions of Bender and Eisenmenger (1941) who concluded from studies of the intake of calcium, magnesuim, nitrogen, phosphorus, and potassium by calciphilic and calciphobic plants that the intake of phosphorus decreased with the addition of calcium. The literature consulted contained no reports of the effect of varying the magnesium and calcium contents of fertilizers upon the phosphorus content of lettuce. However, it is interesting to compare the results obtained in this study with those reported from other investigations.

In his study of the influence of acidity and liming on the growth and chemical composition of lettuce, Crist (1925) found from 0.22 to 0.86 per cent of phosphorus in Michigan lettuce depending upon the fertilization. Zimmerley (1930) concluded from an extensive study of the effects of heavy applications of phosphorus on the growth and composition of lettuce that Virginia lettuce contained from 0.14 to 0.42 per cent of phosphorus. Davidson and LeClerc (1936) determined the variation of mineral content of vegetables and found from 0.59 to 0.98 per cent of phosphorus in different samples of lettuce. McGeorge, Wharton, and Frazier (1940) found approximately 0.5 per cent of phosphorus in lettuce grown in Arizona. This value was also reported by Chatfield and Keiles (1941) for fresh head (bleached) lettuce.

Hydrogen Ion: Commercial and home gardeners have concluded that adding lime to soil improves the quality and yield of lettuce. Accordingly, when the lettuce used in this study was harvested for assay the soil was tested for its hydrogen-ion concentration. The values obtained were Plot A, pH 4.40; Plot B, pH 4.95; Plot C, pH 6.95; and Plot D, pH 7.05. These values are of the same order as those reported by investigators who have given considerable attention to the effect of different soil hydrogen-ion contents upon the growth and quality of lettuce. Crist (1925) studied the growth of lettuce in nutrient solutions with initial pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 and obtained the best plants and maximum growth at pH 5.0. Zimmerley (1930) feels that the optimum conditions for the four seasons are included in the range of pH 5.7 to 7.1. Morgan and Jacobson (1940) are of the opinion that the soil should be limed sufficiently to keep the reaction at pH 6.2 to 6.6.

DISCUSSION

Inspection of the average values reveals that for all practical purposes the lettuce grown under the soil, seed, fertilizer, sunshine, rainfall, and climatic conditions which existed for Plot A contained as much or more carotene, riboflavin, calcium, iron, and phosphorus as lettuce grown on the other similar experimental plots which received supplementary magnesium or calcium or both. The supplementary magnesium sulfate added to Plot B increased the magnesium content of lettuce above that from Plot A. In general, the use of lime as a supplementary fertilizer decreased the iron and magnesium contents of lettuce. This was particularly noticeable for magnesium.

The average values obtained for the 16 samples of lettuce, which were rather typical of the general Boston head lettuce crop of this locality, were 2.7 mg. per 100 grams for carotene, 0.08 mg. for riboflavin, 24.0 mg. for calcium, 2.1 mg. for iron, 13.2 mg. for magnesium, and 23.2 mg. for phosphorus. Such values show that Boston head lettuce, even when devitalized by the removal of the dark green exterior leaves, contains valuable minerals and vitamins. Thus it is very properly classified as one of the valuable "protective foods" for the human dietary.

SUMMARY

A study was made of the effect upon the mineral and vitamin content of Boston head lettuce of supplementing the usual fertilizers with mag-

nesium or calcium salts or both. The lettuce, which was grown under four experimental conditions, was assayed when it had developed to the usual market conditions. All the dark green, exterior leaves ordinarily discarded by the grower, wholesaler, retail grocer, and housewife were eliminated. The pale green or nearly white compact lettuce heads were assayed for water, carotene, riboflavin, calcium, iron, magnesium, and phosphorus. The use of magnesium sulfate as a supplementary fertilizer increased the magnesium content of the lettuce but it also reduced the calcium, iron, and phosphorus content. The use of magnesium sulfate and lime or lime alone as a supplementary fertilizer tended to reduce carotene, calcium, iron, and phosphorus contents of the lettuce and caused a definite decrease in the magnesium content. The values obtained show lettuce to be a valuable source of carotene, riboflavin, calcium, iron, magnesium, and phosphorus and to be well deserving of its classification as a "protective food" for the human dietary.

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A CHROMATOGRAPHIC INVESTIGATION OF THE CAROTENOID PIGMENTS OF THE AVOCADO[1]

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The California avocado is a fruit characterized by an unusually high fat content. In connection with investigations of its nutritional value interest became centered on determining the nature of the pro-vitamin A and other carotenoids in the oil fractions of the avocado. Weatherby, Yontz, and Watson (1929) and Jansen and Donath (1924) demonstrated that avocado contained vitamin A in appreciable quantities, while Stammers (1927) and Bacharach and Smith (1938) have stated that vitamin A is nearly absent. It is the purpose of this study to investigate the carotenoid responsible for any vitamin A activity that avocado oil may have.

Recent developments in the use of chromatography as a method for the isolation and identification of the various carotenoids by the following investigators have suggested the application of chromatography to this study: Zechmeister and Cholnoky (1941); Strain (1938, 1942); Zscheile, White, Beadle, and Roach (1942); and Bickoff and Williams (1943). It was found that owing to the high ratio of oil to pigment in the avocado the direct chromatographic adsorption could not be carried out since the oil rapidly inactivated the adsorbent and so cut down the adsorbing capacity of the column.

EXPERIMENTAL PROCEDURE

In order to eliminate any destruction of carotenoids by light, all of the experimental work was carried out in a darkened room. Necessary light was provided by a 25-watt tungsten bulb in a desk lamp situated so that no light was directed on the carotene solutions.

Chromatographic Separation: One hundred sixty grams of Nabal avocado (edible portion) were weighed into the micro cup of a Waring blender, and blended for one minute with 150 ml. of acetone and 25 ml. of skellysolve F to break down the tissue. Twenty-five grams of Celite 545[2] were added and the mixture was blended for another 30 seconds. The blended mixture was transferred to a Büchner funnel and filtered through a qualitative grade of filter paper with the aid of suction. The pulp was removed and transferred to the blender cup and re-extracted with acetone and skellysolve F, the amount of acetone being decreased and the skellysolve increased with each extraction. Seven extractions were required to remove completely all of the colored pigments from the pulp. The complete extraction required approximately 700 ml. of acetone and 600 ml. of skellysolve F.

[1] Supported by a grant from Avocado Growers of California.

[2] Celite 545 was obtained from Johns Manville Sales Corporation.

The extracts were transferred to a two-liter separatory funnel, and distilled water equivalent in volume to the acetone used was added. Two hundred ml. of saturated sodium sulfate solution was added to decrease emulsions. After standing five minutes the aqueous layer was drawn off and extracted in another two-liter separatory funnel with 50-ml. portions of skellysolve F until the extracts were colorless. Five extractions were required to remove all of the color.

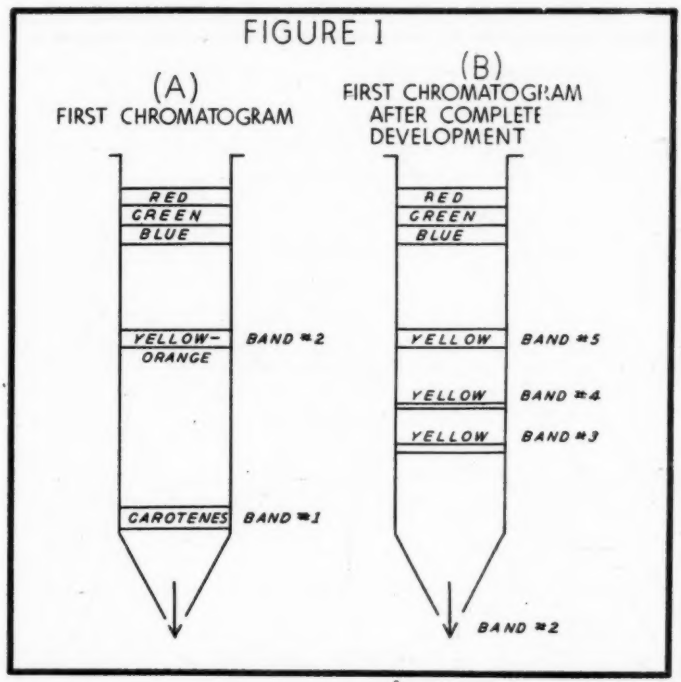
The extracts were combined and washed with distilled water until the washings were free of acetone (salicylaldehyde test). The skellysolve solution of the pigments was dried over anhydrous sodium sulfate and evaporated to 100 ml. with the aid of suction, keeping the solution under an atmosphere of nitrogen.

In order to eliminate the possibility of carotene destruction that occurs in the presence of chlorophyll, according to Pepkowitz (1943), and to remove some of the pigments that are more strongly adsorbed, the blue-green solution was chromatographed on magnesia. A column 35 by 180 mm. was packed with a 3:1 mixture of Celite 545 and magnesia.[3] The mixture was wetted with 15 ml. of skellysolve F, and the pigment solution was poured onto the column. The green pigments were strongly adsorbed and adhered to the first 50 mm. of the column. Below the green was a yellow zone which moved rapidly down the column. After all of the pigment solution was transferred to the column, it was washed with pure skellysolve H, taking care to have the adsorbent covered with solvent at all times. The column was washed with a total of 200 ml. of skellysolve H, after which it had the appearance shown (Fig. 1A). The carotenes (Band 1) were washed out into the filtrate with another 100 ml. of skellysolve, containing three per cent acetone. The chromatogram was then developed further with skellysolve H containing 10 per cent of acetone. This solvent eluted the yellow band No. 2 (Fig. 1A) which passed into the filtrate. Three new yellow bands developed and the column had the appearance shown (Fig. 1B). The filtrate containing band No. 2 (Figs. 1A and 1B) was set to one side for further study, and the adsorbent was extruded from the column by the application of five to 10 pounds of pressure from a nitrogen cylinder, Turkevich (1942). The adsorbent containing band No. 5 (Fig. 1B) was cut out and transferred to a glass-stoppered flask containing acetone. The other colored bands were discarded.

Isolation of the Carotenes: The filtrate containing the carotenes (Fig. 1A) was evaporated *in vacuo* to 100 ml. maintaining an atmosphere of nitrogen over the carotene solution. Any fat remaining in the filtrate was saponified by boiling the 100 ml. of residue under reflux for 30 minutes with 100 ml. of 10-per cent ethanolic KOH. The saponification mixture was cooled, 150 ml. of water were added, and the pigments were extracted with skellysolve F. The skellysolve solution was washed three times with water, once with 0.5N KOH, and then with water until the washings were neutral to phenolphthalein. The carotene solution was dried over anhydrous sodium sulfate and evaporated to 50 ml. using the same precautions

[3] Adsorptive powdered magnesite No. 264 was obtained from Westvaco Chlorine Products Corporation.

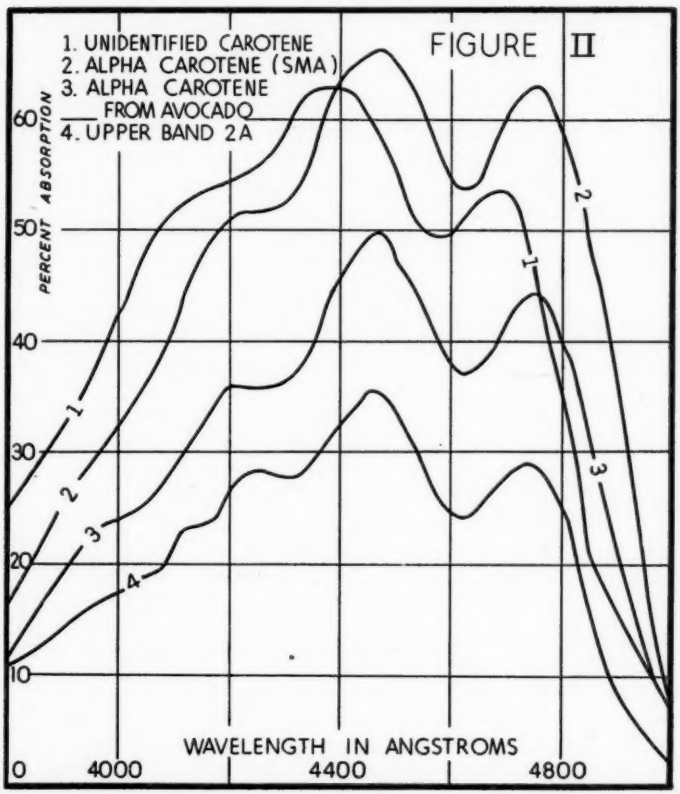
as before to avoid carotene destruction. The solution was chromatographed on a 1:1, celite-magnesia column 20 by 200 mm. Yellow pigments were adsorbed on the top two centimeters of the column, and below this were the carotene bands. The development was carried out with pure skellysolve H, the bands of a and b carotene being clearly defined. After proper development the column had the appearance described below. At the top was a diffuse yellow zone extending for 30 mm.; halfway down the column was a bright lemon yellow band followed at 25-mm. intervals



by beta and alpha carotene, respectively. The adsorbent was extruded from the tube and the bands of alpha, beta, and unknown carotene were mechanically separated. The pigments from each band were eluted with acetone and separated from the adsorbent by filtration through sintered glass funnels. The acetone solution of carotene was transferred to a separatory funnel, 10 ml. of skellysolve B was added, and the carotene transferred to the skellysolve by the addition of water. The skellysolve solution was washed free of acetone, the alpha carotene was rechromatographed on 1:1, celite and magnesia column, and the beta carotene was chromatographed on an alumina [4] column. The unknown carotene was diluted to 25 ml. in a volumetric flask with skellysolve B. The alpha carotene was

[4] Activated alumina Minus 80, chromatographic grade was obtained from the Aluminum Ore Company, East St. Louis, Illinois.

considered pure as shown by the fact that only one band could be seen even after extensive development. The band was cut out as before and eluted. The pigment was made up to 10 ml. in a volumetric flask. The beta carotene gave only one band on alumina; the neo-beta carotene found by Beadle and Zscheile (1942) was not present or, if present, was in such small quantities that the band could not be detected. Transmission values for the three pigments were obtained with the Beckmann spectrophotometer and the values were plotted. The transmission curve of the un-

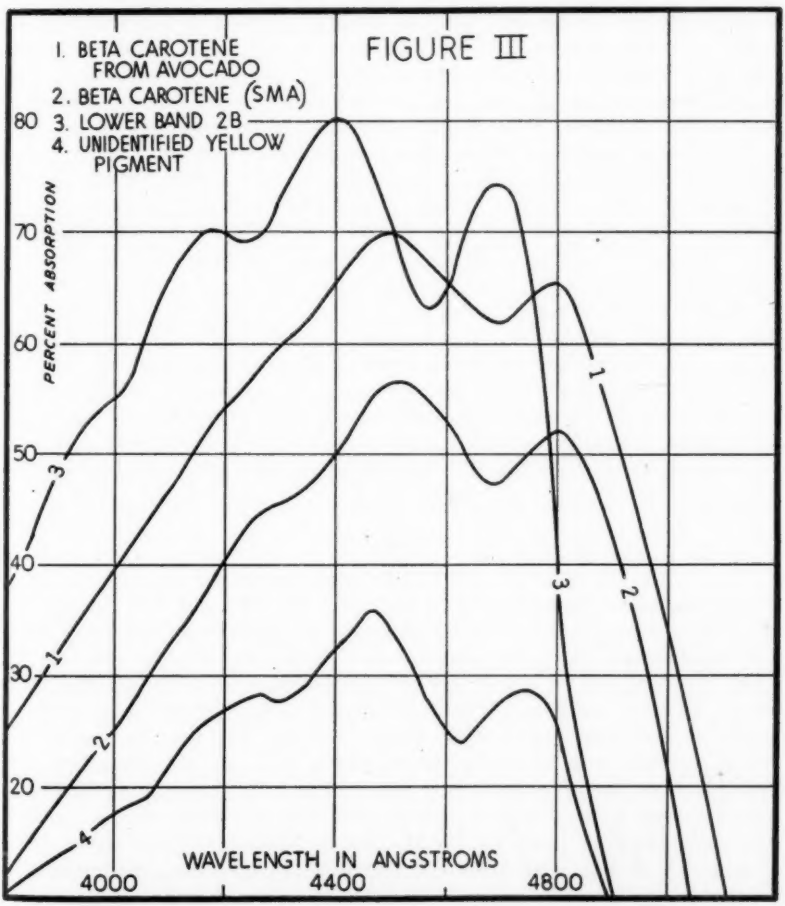


known carotene was shown (Fig. 2, Curve 1); spectral curves of alpha and beta carotene were shown (Fig. 2, Curves 2 and 3) and (Fig. 3, Curves 1 and 2) together with transmission curves of alpha and beta carotene obtained from the SMA Corporation.

The pigment solution from band No. 2 (Fig. 1A) was washed free of acetone, dried over anhydrous sodium sulfate, and evaporated to 100 ml. with the usual precautions. A column 20 by 160 mm. was packed with 1:2

mixture of Celite 545 and lime.[5] The column was wetted with 15 ml. of skellysolve H, and the pigment solution was chromatographed. The pigments were strongly adsorbed on the top three centimeters of the column, and the development was carried out with skellysolve H containing five per cent of acetone. Two bands gradually developed and were separated by three cm. by the time the pigments reached the bottom of the column. The adsorbents were extruded and the bands were carefully separated and eluted with acetone in the manner described before. The transmission curves of the two pigments were obtained with the Beckmann spectrophotometer and were reproduced (Fig. 3, Curve 3 and Fig. 2, Curve 4). The shape of the spectral and the location of their bands on the chromatographic column makes the authors assume that these pigments represent some of the xanthophylls.

[5] Shell Brand lime, chemical hydrate; 98 per cent through 325 mesh.



The acetone solution of band No. 5 (Fig. 1B) was transferred to a separatory funnel, and the pigments transferred to skellysolve H. After being washed free of acetone the skellysolve solution was dried over anhydrous sodium sulfate and chromatographed on a 20 by 250 mm. column packed with 1:2 Celite 545 and lime mixture. The pigment solution was poured onto the column and developed with skellysolve H containing 15 per cent of acetone. The pigments separate into a diffuse zone followed by a sharp yellow band located three cm. from the bottom of the column. The adsorbent was extruded from the column and the lower band was separated. The pigment was eluted in the usual manner, separated from the adsorbent, and the pigment was taken up in skellysolve B. Spectral transmission values were obtained on the Beckmann spectrophotometer and the curve (Fig. 3, Curve 4) was plotted. The pigment could not be identified but undoubtedly represents a part of the xanthophyll fraction, which is still impure.

Quantitative Determinations: The edible portion of the avocado was weighed into an Erlenmeyer flask and saponified by boiling for 30 minutes with 100 ml. of 10-per cent ethanolic KOH. The saponified mixture was filtered on a sintered glass funnel and the residue was washed alternately with 25-ml. portions of skellysolve F and ethanol until the filtrate was colorless. The combined filtrates were transferred to a separatory funnel and 100 ml. of distilled water were poured through the solution. The aqueous-alcoholic layer was drawn off and re-extracted with skellysolve F until the extracts were colorless. The skellysolve extracts were combined and washed three times with water, and the xanthophylls were then removed by washing with 90-per cent methanol. The washing with methanol was continued until no more color was extracted. The skellysolve solution was dried over anhydrous sodium sulfate and evaporated to 50 ml. *under vacuo*. Crude carotene was determined on an aliquot of this solution, the remainder being used for chromatographic separation. One aliquot of the carotene solution was chromatographed on a magnesia column, as suggested by Kemmerer (1942), and the bands of alpha and beta carotene separated and quantitatively determined. In another aliquot total carotene was determined by the method suggested by Wall and Kelley (1943).

RESULTS

The crude carotene of the Fuerte avocado determined by the above method on two different samples amounted to 2.27 and 2.36 micrograms of carotene per gram of avocado. The Nabal avocado was found to contain 2.35 micrograms of crude carotene per gram. The Fuerte avocado was found to contain 0.54 microgram of beta carotene and 0.06 microgram of alpha carotene per gram. The Nabal avocado was found to be lower in alpha and beta carotene than the Fuerte variety. The beta carotene of the Nabal avocado was 0.40 microgram per gram and the alpha carotene amounted to 0.02 microgram per gram. The total carotene calculated as beta carotene by the method of Wall and Kelley (1943) gave a value of 0.75 microgram of carotene for the Nabal avocado. From the work of Weatherby, Yontz, and Watson (1929) it appears that the vitamin A content of the avocado is approximately 0.6 international unit per gram;

this is in fair agreement with our determinations in which we found nearly an equivalent amount of beta carotene, the amount of alpha carotene as shown above being very insignificant.

SUMMARY

Alpha and beta carotene have definitely been identified as constituents of the avocado. An unknown carotene has been found in avocado and the spectrophotometric transmission curve is presented. Three unknown pigments were isolated and the transmission curves determined. The vitamin A activity found by others can be satisfactorily accounted for by the carotenes isolated.

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CAROTENE AND CHLOROPHYLL CONTENT OF FRESH AND PROCESSED SWISS CHARD AND BEET GREENS[1]

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This is the second paper in a series presenting the effects of processing on the vitamin content of "greens." It describes specifically the carotene and chlorophyll content of the leaves of certain varieties of the beet species (*Beta vulgaris*) as affected by marketing, home cooking, and drying.

The literature relating to the carotene content of the leaves of the beet family and the effects of different types of processing upon its concentration is limited. Booher and Marsh (1941a), using rat-growth methods, found that beet greens (Wonderful Early Spring) contained 16,100 I. U. of vitamin A value per 100 grams of fresh leaves and that chard contained 14,500 I. U. Sheets, Leonard, and Gieger (1941), using the Hegsted, Porter, and Peterson method, found that Swiss chard (Fordhook Giant) contained 51.6 mg. of carotene per 100 grams dry weight in the blade and 2.6 mg. in the midrib; Swiss chard of the Lucullus variety, 46.1 mg. carotene in the blade and 2.4 mg. in the midrib; while in garden-beet leaves, there were 57.2 mg. carotene in the blade and 10.3 mg. in the midrib.

It is frequently stated that there seems to be a direct parallel between greenness, or chlorophyll content, and vitamin A activity. A number of years ago this relationship was observed by Steenbock and Sell (1922) who, by biological tests, found vitamin A to be higher in the green than in the white leaves of cabbage. Dye, Medlock, and Crist (1927) found green lettuce leaves superior in vitamin A value to the blanched leaves. More recently Johnson and Miller (1940) obtained significant correlations between the concentration of total chlorophylls and either total carotenoids or beta carotene in forage grasses.

In general, literature indicates that there is no great loss of carotene owing to ordinary cooking processes. Coward and Morgan (1935) found no loss of vitamin A potency when cabbage, carrots, and beans were cooked. Newton (1931) found no loss when turnip greens or collards were boiled for 45 minutes. Other investigators—Booher, Hewston, and Marsh (1941b) on cooking leafy vegetables and green beans; MacLeod and Utley (1937) and MacLeod (1939) on cooking sweet potatoes; and Richardson and Mayfield (1940) on cooking peas—have found more carotene in the cooked product than in the raw. Oser, Melnick, and Oser (1943) found, in the case of peas, a 10-per cent loss of carotene when they were cooked in a

[1]Approved by the director of the Michigan Agricultural Experiment Station for publication as Journal Article No. 688 (n.s.).

small amount of water and approximately an 18-per cent loss when cooked in a large amount of water.

Little has been published regarding the effect of drying on the carotene content of vegetables, but the general opinion is that much greater losses occur with this than with other types of processing. Fraps and Treichler (1933) reported an 80-per cent loss of carotene in vacuum drying of raw carrots, 29 per cent with sweet potatoes, and 65 per cent with vacuum-dehydrated spinach. De Felice and Fellers (1937) determined that fresh and dehydrated spinach were 718 I. U. and 495 I. U. in vitamin A value per gram, respectively. If moisture were taken into consideration this would be approximately a 93-per cent loss. Moyer (1943) found that there was little loss of carotene from rutabagas during the drying processes but that at all storage temperatures above —40°C.(—40°F.) its loss was rapid. This loss decreased with storage under carbon dioxide. Cruess (1943) reported that blanched spinach lost approximately 27 per cent of its carotene when dried at 71°C.(159.8°F.) for four hours and 11 per cent when the temperature was 82°C.(179.6°F.) and drying time three hours. In studying the effect of refrigerated storage on the carotene content of lettuce, Harris and Mosher (1941) reported that in the preservation of this vitamin high humidity and low air movement are important factors and that the rate of carotene destruction during dehydration is apparently linear until about 40 per cent of its weight is lost, then the destruction appears to be accelerated.

EXPERIMENTAL PROCEDURE

Leaves of four horticultural varieties of the beet family (*Beta vulgaris*) were selected for study and included the common table beet (Detroit Dark Red), the sugar beet (U. S. 200 x 215), and two types of Swiss chard (Fordhook and Rhubarb). Complete descriptions of the experimental growing, harvesting, and processing of the greens are given in the first paper of this series by Porter, Schlaphoff, Wharton, Briant, and Beltz (1944). Briefly, two methods of *home cooking* were employed: (a) using a moderate amount of rapidly boiling water and (b) using only that water which remained clinging to the leaves after the last rinsing. *Institution preparation* consisted of cooking large lots of greens in a steam-jacketed kettle and then holding them for either 10 minutes or 60 minutes on the steam table. *Home drying* was accomplished in a homemade drier used on a gas stove. The dried leaves were prepared for serving by cooking until tender in boiling salted water equivalent to 25 times the weight of the dried greens.

The chlorophyll and carotene were determined by the Petering, Wolman, and Hibbard procedure (1940) using a Waring Blendor for fine dispersion of the plant material in absolute alcohol. The standard curve was made using carotene which was 90 per cent beta and 10 per cent alpha carotene (S.M.A. Corp.) The chlorophyll for the standard curve was prepared by Dr. C. L. Comar, Experiment Station Chemistry, Michigan State College. Chlorophyll readings on fresh plant extracts, using the method of Petering *et al.* (1940), and the standard so prepared, were checked spectrophotometrically by Dr. Comar and found to agree completely.

From each lot of greens that was processed, an aliquot of the freshly picked leaves was selected as a control. After processing, each composite sample was analyzed in triplicate. Moisture content was determined on triplicate samples from all aliquots by drying at 95 to 105°C. (203 to 221°F.) for 24 hours. When the number of samples permitted, mean vitamin values were analyzed by variance methods for statistically significant differences. Correlation between carotene and chlorophyll contents of raw leaves was also studied.

RESULTS AND DISCUSSION

An analysis of variance in the carotene and chlorophyll data for the fresh leaves (Table 1) showed no significant differences in the levels of carotene and chlorophyll in leaves picked in August and in September. Therefore, the results of the harvestings made in the two months have been considered together.

TABLE 1

Carotene and Chlorophyll Content of Freshly Picked Greens

Variety of greens	Number of samples	Moisture	Carotene		Chlorophyll	
			In fresh leaves	Calculated to dry basis	In fresh leaves	Calculated to dry basis
		gm./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Swiss chard						
Fordhook	24	91±0.2	5.8±0.1	62±1.3	135±4.3	1448±42.7
Rhubarb	24	89±0.3	6.1±0.2	58±1.2	141±4.3	1332±33.9
Beet leaves						
U. S. 200 x 215	6	88±0.3	6.4±0.2	52±1.1	144±3.8	1171±20.0
Detroit Dark Red	6	90±0.3	5.5±0.2	55±1.1	135±2.5	1358±23.7

All varieties of leaves contained approximately six mg. of carotene per 100 grams of fresh leaves. The chlorophyll levels, however, ranged from 135 to 144 mg. per 100 grams of fresh sample, a ratio in the neighborhood of 24:1 with carotene. There was a correlation between the carotene and chlorophyll content of the leaves, significant at the one-per cent level, which coincides with the popular idea that the carotene content increases with greenness and also with the findings of Johnson and Miller (1940) on different varieties of grasses.

The effects of two different methods of home cooking on the carotene content of greens are presented (Table 2) and those of institutional-cooking procedures (Table 3). The carotene concentration was not much changed by either home-cooking method, although, in most cases, the carotene content of the cooked greens was greater than that of the raw when compared on a dry basis. These differences were not statistically significant. Institution-cooked greens (eight-lb. lots), whether held for 10 or 60 minutes on the steam table, contained more carotene than the raw when compared on the dry-weight basis, these apparent increases in the institution-cooked greens being significant (statistically) only in the case of the Rhubarb variety of Swiss chard. As mentioned in the previous paper by Porter *et al.* (1944), the institution cooking was not as well controlled as was desirable, since a period of 20 to 30 minutes for the transfer of the cooked

TABLE 2

Effect of Home-Cooking Methods on Carotene Content of Greens[1]

Variety and period	Fresh raw sample (dry wt.)	Method 1[2]				Method 2[3]			
		Moisture	Carotene values			Moisture	Carotene values		
			As served	Dry	Change from fresh values		As served	Dry	Change from fresh values
Swiss chard	*mg./100 gm.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Fordhook									
Period 1	65±1.3	92±0.6	5.1±0.4	61±3.9	—6.2	90±0.3	6.6±0.4	63±1.3	—3.1
Period 2	60±1.8	91±0.3	6.4±0.4	71±4.6	18.3	89±0.2	6.7±0.3	60±2.5	0.0
Rhubarb									
Period 1	60±1.8	90±0.1	7.1±0.6	74±5.9	23.3	88±0.5	7.4±0.3	62±3.6	3.3
Period 2	55±2.4	91±0.3	6.1±0.4	68±2.3	23.6	89±0.4	6.6±0.3	59±2.4	7.3
Beet leaves									
U. S. 200 x 215	52±1.1	90±0.3	6.3±0.2	62±2.9	19.2	86±0.3	6.8±0.4	49±3.0	—5.8
Detroit Dark Red	55±1.1	93±0.2	5.1±0.5	73±7.0	32.7	89±0.2	6.1±0.2	53±2.1	—3.6

[1] Each figure represents an average of six determinations. [2] Cooked in a moderate quantity of rapidly boiling water. [3] Cooked in water clinging to the leaves after the last rinsing.

TABLE 3

Effect of Institutional-Cooking Methods on Carotene Content of Greens[1]

Variety and period	Fresh raw sample (dry wt.)	Method 1[2]				Method 2[3]			
		Moisture	Carotene values			Moisture	Carotene values		
			As served	Dry	Change from fresh values		As served	Dry	Change from fresh values
Swiss chard	*mg./100 gm.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Fordhook									
Period 1	65±3.2	90±0.2	7.3±0.5	75±4.7	15.4	89±0.6	7.9±0.6	69±4.5	6.2
Period 2	59±3.2	92±0.2	5.4±0.2	63±3.0	6.8	90±0.2	5.6±0.4	55±4.8	—6.8
Rhubarb									
Period 1	57±2.2	90±0.3	8.1±0.5	80±4.5	40.4	88±0.4	8.1±0.5	68±4.0	19.3
Period 2	60±3.0	91±0.2	6.1±0.2	69±3.3	15.0	91±0.2	7.3±0.2	76±2.0	26.7
Beet leaves [4]									
U. S. 200 x 215	50±1.4	87±0.3	6.7±0.3	53±0.3	6.0	88±0.2	7.1±0.4	58±3.1	16.0
Detroit Dark Red	53±1.6	91±0.1	5.5±0.2	57±2.1	7.5	90±0.4	5.9±0.2	58±0.9	9.4

[1] Each figure represents an average of six determinations. [2] Held on steam table 10 minutes. [3] Held on steam table 60 minutes. [4] Beet leaves, U. S. 200x215, were cooked for 16 minutes and Detroit Dark Red for 12 minutes in a pressure steamer. The steam-table treatment was the same as that for the Swiss chard.

material from the institution kitchen to laboratories may have represented a critical period so far as the vitamin contents of the greens were concerned. The gains in carotene observed after processing leafy material, while reported by other investigators as well as in this study, are believed by the writers to be more apparent than real. Further study on this problem is being continued in this laboratory and will be published later.

Uniform sampling of the plant material, such as Swiss chard, was difficult owing to the large midrib. Sheets *et al.* (1941) showed that the leaf blade furnished 96.3 to 99 per cent of the total carotene of the leaf. Since it was desired in this study to learn the nutritive value of the greens as they were served on the table, the whole leaf (blade and midrib) with only the petiole removed was analyzed. This accounted for part of the experimental error because in taking relatively small portions for analysis, even from a well-mixed composite sample, it was entirely probable that an equitable distribution of blade and midrib was not always obtained. Further, variations in the water content of the different structures of the leaf decreased uniformity of sampling.

The carotene determinations on greens shortly after drying, after the newly dried leaves were cooked for serving, and then after six and seven months of storage at 18°C.(64.4°F.) are shown (Table 4). Initial drying caused an 18- to 30-per cent loss of carotene. When the dried greens were cooked and prepared for serving, however, they gave carotene values which compared favorably with those of the fresh raw sample, these values being calculated on the dry-weight basis. Storage for six to seven months increased carotene losses to more than 60 per cent. It appeared then, that while initial drying losses were not too serious and were within the range of such losses as observed by Cruess (1943), the method did not produce a vegetable that could be relied upon as a dietary source of carotene, especially if stored under ordinary home conditions.

Marketing as practiced in this experiment represented conditions similar to those used in a local market and caused no significant change in carotene content (Table 5). The leaves were not wilted after 24 hours of storage in a moist burlap wrapping. Harris and Mosher (1941) found the distribution of carotene in lettuce to be correlated with the percentage loss in moisture and that the degree of "wilting" may be used as an index of loss of the initial carotene content.

SUMMARY

A study was made of the carotene content of leafy vegetables as affected by (1) variety; (2) home cooking by two widely used home-cooking methods; (3) "holding" the greens cooked by an accepted institutional method for 10 minutes or 60 minutes on a steam table; (4) home drying and storage under home conditions; and (5) marketing as on the local farmers' market. The varieties of leaves were all from the beet species (*Beta vulgaris*): the common table beet (Detroit Dark Red), the sugar beet (U. S. 200 x 215), and two varieties of Swiss chard (Fordhook and Rhubarb). The beet leaves were analyzed in July and the Swiss chard at two periods in August and in September. Chlorophyll determinations were made on all

TABLE 4

Effect of a Home-Drying Method on Carotene Content of Greens

Variety and period	Number of samples	Moisture	Newly dried leaves			Moisture	Cooked, newly dried leaves		Stored, dried leaves	
			Carotene values				Carotene values			
			Freshly picked (dry basis)	Dried (dry basis)	Change from fresh values		Cooked (dry basis)	Change from fresh values	Dried and stored (dry basis)	Change from fresh values
		gm./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*gm./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*pct.*
Swiss chard										
Fordhook										
Period 1	6	7.3±0.4	65±1.3	51±1.8	—22	86±0.4	64±3.4	—1.5	23 (7 mo.)	—65
Period 2	3	7.9±0.1	60±3.9	44±1.7	—28	89±0.2	60±2.8	—1.6	22 (6 mo.)	—64
Rhubarb										
Period 1	6	10.0±0.2	57±0.5	40±2.9	—30	87±0.6	67±0.8	17.5	.....	.....
Beet leaves										
U. S. 200 x 215	3	10.3±0.1	51±2.8	42±1.2	—18	86±0.3	56±1.7	9.8	.....	.....
Detroit Dark Red	3	6.0±0.01	56±0.9	45±1.6	—20	87±0.3	54±2.7	—3.6	.....	.....

the fresh samples and compared with the carotene concentration of the same sample.

The average carotene content for each variety of leaves was six mg. per 100 grams of fresh material, while the chlorophyll content ranged from 135 to 144 mg. There was no significant difference in the carotene or chlorophyll concentrations between the August and September harvestings of Swiss chard. The correlation of the carotene to chlorophyll content was highly significant.

TABLE 5

Effect on Carotene Content of Holding Greens for 24 Hours Under Local Market Conditions

Variety and period	Moisture of "held" leaves	Carotene		
		Freshly picked (dry basis)	Held 24 hours (dry basis)	Change from fresh values
Swiss chard				
Fordhook	*gm./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*
Period 1	91±0.1	56±3.5	66±3.3	17.9
Period 2	92±0.1	60±1.8	54±2.7	—10.0
Rhubarb				
Period 1	90±0.3	61±2.4	57±2.0	—6.6
Period 2	91±0.5	55±2.4	64±3.3	16.4

Neither method of home cooking significantly affected the carotene content of the leaves, although there were apparent gains in carotene, particularly in the Rhubarb chard. Institution cooking and holding on the steam table for 10 or 60 minutes did not significantly affect the carotene content of the Fordhook but caused a highly significant gain in the Rhubarb Swiss chard. The home-drying method caused an 18- to 30-per cent loss of carotene and storage for six to seven months increased this loss to more than 60 per cent. The method of marketing did not significantly affect the carotene content.

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CELLULAR CHANGES IN CERTAIN FRUITS AND VEGETABLES DURING BLANCHING AND DEHYDRATION

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The keeping qualities of dried foods vary greatly. Some high-sugar fruits, such as raisins, dates, figs, and prunes, keep well when simply dried in the sun; this process is now in general use. Other fruits (apricots, peaches, nectarines, apples, and pears) have commonly been held in the fumes of burning sulfur from one to many hours before being placed in the sun—Long, Mrak, and Fisher (1940). Sulfuring gives the fruits a translucent pleasing appearance, retards chemical changes and thus preserves the products, repels insects and prevents fermentation during drying. Trays of sulfured fruits, after a partial sun-drying, are usually stacked so that the fruit may continue to dry without direct exposure to the rays. This method tends to preserve the natural colors and flavors.

In sun-drying of cut fruits the halves are commonly placed on the trays with the cut sides up, the pit cavities forming small cups. They are then kept in the fumes of burning sulfur (SO_2) for three hours to overnight, and the sulfur houses often run as high as 26.7 to 37.8°C.(80 to 100°F.). Under these conditions the tissues are killed, sap is released, and often the cups formed by the seed cavities are partially filled with liquid when the fruit is placed in the sun. The fruit acquires a water-soaked or "cooked" appearance.

On trays in the sun, drying fruit often is subjected to well over 100°F., and the temperature fluctuates from day to night. Under these conditions cell walls soften somewhat and the fruit assumes a bright, translucent appearance. If the trays are stacked as soon as this condition is reached and the drying is completed in the shade, a sun-dried product of the highest quality is obtained. During sun-drying, however, the fruit is subject to contamination with dirt, infestation by insects, and some loss of color and flavor by oxidation.

In the present search for new and better methods of food preservation, innumerable chemical and physical treatments are being tested. Undoubtedly many of these will ultimately find a place in commercial processes. Of the various procedures now used in preparing fruits and vegetables for drying, two stand out—sulfuring and precooking or blanching. Since these treatments have proved their worth, according to Mrak, Phaff, Fisher, and Mackinney (1943) and Reeve (1942), it seems desirable to understand them clearly in order to control them properly and to utilize every advantage that they present. Furthermore, one should recognize any interrelation between them in order to use each at its highest efficiency.

Blanching enables one to produce a dehydrated fruit of appearance equal or superior to sun-dried; in experiments it has given a product that

retains more of the full flavor of tree-ripened fruit; and dehydration preceded by steam-blanching can be carried out under optimum conditions of cleanliness. It is a common practice also in the preparation of vegetables for canning—Adam and Stanworth (1941). It is becoming popular in dehydration of both fruits and vegetables. Except, possibly, with onions, it is beneficial in preventing vitamin loss; it inactivates enzymes, thus preventing deterioration in storage—Reeve (1943a, b); and it improves the appearance of the finished product. The investigations here described were undertaken in order to explain why blanching improves the appearance of both fruits and vegetables that have been dehydrated. It was also hoped that the superior keeping qualities of blanched products might be somewhat elucidated.

EXPERIMENTAL PROCEDURE

Most of the cytological studies to be described were started with fresh fruits and vegetables from orchards and gardens or from local stores. In the laboratory these were sectioned by hand and examined under the microscope. By having both transmitted and reflected light, it is easy to determine the general nature of the tissues, the amount of pigmentation, and the amount of intercellular air. A Greenough-type dissecting binocular was used for the preliminary survey of tissue types. In the detailed studies, a Zeiss compound binocular was utilized. The photomicrographs were made by means of an Eastman kodak precision enlarger with microscope attachment and 35 mm. Kodachrome adapter. Negatives were on Agfa Finopan film.

Studies on blanching and drying involved the use of a hot stage through which steam could be passed. A small, round, open-topped, glass cell was sealed into openings in a brass jacket having inlet and outlet tubes. To observe the blanching process, a section of tissue was transferred to a cover slip; this was placed on top of the glass cell, the tissue hanging from the underside. As steam was passed around the cell, the temperature rose rapidly, heating the tissue under observation. A saturated atmosphere was maintained by keeping a thin layer of water in the bottom of the cell.

To observe drying, the tissue was simply mounted on top of the cover slip; and steam was passed through the jacket to warm the tissue which, being exposed to the air, dried at a rate governed by the temperature. The hot stage was used on top of the ordinary stage of a compound microscope with the substage condenser removed. Illumination was obtained from a microscope lamp focused to give a beam of parallel light.

OBSERVATIONS ON FRESH TISSUES

Cells making up the bulk of the edible portions of most fruits and vegetables are parenchyma, that is, relatively unspecialized cells ranging in form from spherical to polyhedral and from approximately isodiametric to greatly elongated or flattened. Intercellular spaces are usually prominent in parenchyma and may occupy an appreciable portion of the total space. Reeve (1942) has described parenchyma cells of vegetable tissues in detail.

A hand section of the flesh of Royal apricot (Fig. 1) shows cells somewhat elongated; intercellular spaces, shown in black because of diffraction of light, are present between the corners and edges of the cells. Such air-filled intercellular spaces, when viewed by reflected light by means of a dissecting binocular or by side illumination with a compound microscope, look like glistening air bubbles against a black background. It is light reflected from such intercellular air masses that gives the flesh of the apple and the pear a characteristic glistening whiteness. The appearance of the fresh flesh of apricot and peach is modified by the color of the carotenoid pigments in the cells. The flesh of the prune, on the other hand, has very large cells but small, inconspicuous intercellular spaces.

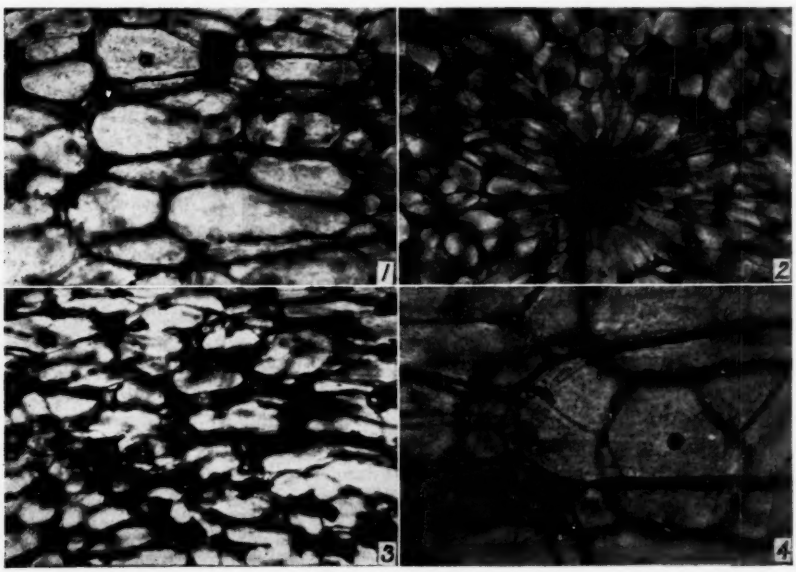


FIG. 1. Hand section of fresh tissue of Royal apricot, showing thin-walled parenchyma cells and prominent air-filled intercellular spaces. ×95.

FIG. 2. Hand section of fresh tissue of Bartlett pear with a center group of stone cells and radiating from it a layer of elongated parenchyma cells. Cells farther from the stone-cell group are roughly isodiametric. Elongated parenchyma also occurs along the vascular bundles and near the core. ×84.

FIG. 3. Hand section of Muir peach where the large proportion of the section is occupied by air-filled intercellular space. ×95.

FIG. 4. Hand section of the fresh tissue of French prune, showing cells that are very large and intercellular spaces that are relatively small. ×95.

Such tissue appears translucent, not unlike cooked apple or pear. Prune cells also contain some pigment, though less than apricot or yellow peach.

The fresh tissue of pear (Fig. 2) shows a dark mass in the center which is a group of stone cells. Such groups are found dispersed throughout the flesh of the pear, giving it its gritty character—Crist and Batjer (1931) and Smith (1935). These stone-cell groups are very numerous

just below the epidermis and also around the core. They are made up of small cells having thick, heavily lignified walls. They differ in number and size according to the variety, being fewer and smaller in the Bartlett pear than in the winter varieties.

Surrounding the stone-cell group is a layer of much elongated parenchyma. This tissue in turn is surrounded by ovoid and spherical parenchyma cells having very thin walls and prominent intercellular spaces. The Muir is the most popular peach variety for drying. Addoms, Nightingale, and Blake (1930) have described the development of the peach in detail. In the flesh of the Muir (Fig. 3) prominent intercellular spaces make up an interconnected, air-filled phase that appears to occupy at least 10 per cent of the total tissue volume.

In contrast to these three fruits the cells of the French prune, the popular drying variety (Fig. 4), are very large, and intercellular air is much less conspicuous. The prune is not commonly sulfured, the only pretreatment used being a brief dip in hot water or dilute lye to crack the cuticle. This treatment opens the skin and allows more rapid diffusion of moisture from the drying fruit.

The fleshy tissues of root vegetables, such as carrot, turnip, and sweet potato; the tubers of white potatoes; and the stems or petioles of cabbage, broccoli, and other vegetables are largely composed of parenchyma cells not unlike those pictured in the fruits. Besides this simple, unspecialized parenchyma all these fruit and vegetable structures have vascular tissues with a variety of specialized cell types. In high-quality fruits and vegetables, however, the vascular tissues are small in amount and contain little lignified wood or fiber tissue; they constitute the "strings" or "fibers" of the preserved product. Blanching tends to soften the vascular tissues and renders them less conspicuous in the final cooked food.

EFFECTS OF STEAM-BLANCHING

In histology it is common practice to evacuate tissues in order to displace intercellular air with the killing fluid. The tissues assume a translucent, water-soaked appearance and usually sink beneath the surface of the fluid.

Another way to displace intercellular air is to heat the tissues. This causes the air to expand, forcing it out just as evacuation does. If the tissues are in water, the gases in the intercellular spaces contract upon cooling, and the liquid is drawn in. Again, cellular tissues upon cooling usually assume a translucent appearance and sink beneath the surface. In cooking, the cells, which initially were turgid, are rapidly killed. Sap, pressed from them, is available to displace intercellular air. Softening of the cell walls further alters the inherent structural properties of the tissue, allowing the cells to distort and to fit more closely together.

Steam-blanching has the same effect as cooking, with the one difference that the tissues are not surrounded by water. If intercellular spaces are to be completely filled with liquid after steam-blanching, the volume of sap released from killed cells plus the water condensed on the tissues from steam must suffice to occupy the requisite space. Heating must continue long enough to bring about a fairly complete replacement of

intercellular air by water vapor. Unless these conditions are fulfilled air bubbles in the tissues do not completely disappear upon cooling but, during dehydration, are trapped in the tissues and remain indefinitely.

These statements are based upon a thorough study of the blanching process by means of the hot-stage microscope described above. When a section of pear, apple, carrot root, or cabbage petiole tissue is placed on a cover slip and mounted in the cell of the hot stage, as the temperature rises the air bubbles visible throughout the tissue are seen to expand slightly, and very soon small bubbles can be observed rising from the cut ends of intercellular spaces. With continued heating small bubbles move about through the intercellular space system, and sap released from killed cells replaces intercellular air in the finer passages. For this reason, tissue subject to prolonged heating has little or no gas in the finer passages; the bubbles that remain are approximately spherical and occupy the larger intercellular spaces. Filling of the small intercellular spaces with liquid takes place in a jerky fashion as if the external cell surfaces wet with difficulty.

Upon cooling, the bubbles present in the tissue contract rapidly. If heating has been sufficient to replace the air rather completely with water vapor, the bubbles will contract completely and disappear. If the air has not been completely replaced, bubbles will persist but will be reduced in size. If they are small enough, they will rather rapidly disappear as a result of the pressure upon the gas caused by surface tension of the liquid-gas interface. Such pressure forces the gases into solution and they diffuse outward, finally reaching equilibrium with the atmosphere.

Besides these simple physical effects upon intercellular air, heating with steam causes rapid death of the cells. This is evidenced by change in the appearance of the protoplasm as the proteins are coagulated. Often the pigment bodies, where present, rapidly aggregate. Starch, if present, gels as soon as the gelation temperature is attained. Undoubtedly there are chemical changes occurring that cannot be readily detected with the microscope. For instance Mrak, Phaff, Fisher, and Mackinney (1943) showed that peroxidase is inactivated by steam-blanching; numerous tests have shown that oxidase and catalase are inactivated also. According to Zscheile, Beadle, and Kraybill (1943), blanched vegetables retain more carotene during low-temperature cold storage than unblanched. Other evidences of chemical changes are an enhanced absorption and retention of SO_2—Mrak, Phaff, Fisher, and Mackinney (1943)—and a softening of the vascular bundle tissues in certain cases.

EFFECTS OF DRYING

By gently heating sections of tissues in the open air on a cover slip, using the hot stage described, one can observe the changes that attend drying. Starting with fresh apple or pear tissue, one first notices the glistening surface of the freshly cut cells and observes that the whole cell mass is impregnated with liquid. As drying proceeds, this surface film of moisture gets thinner and finally disappears from the convex surfaces, leaving them dull gray. Meanwhile, under the influence of forces resulting from water loss, bubbles within the intercellular spaces expand,

and to-and-fro movement of small columns of liquid may be observed as more and more air enters the tissue.

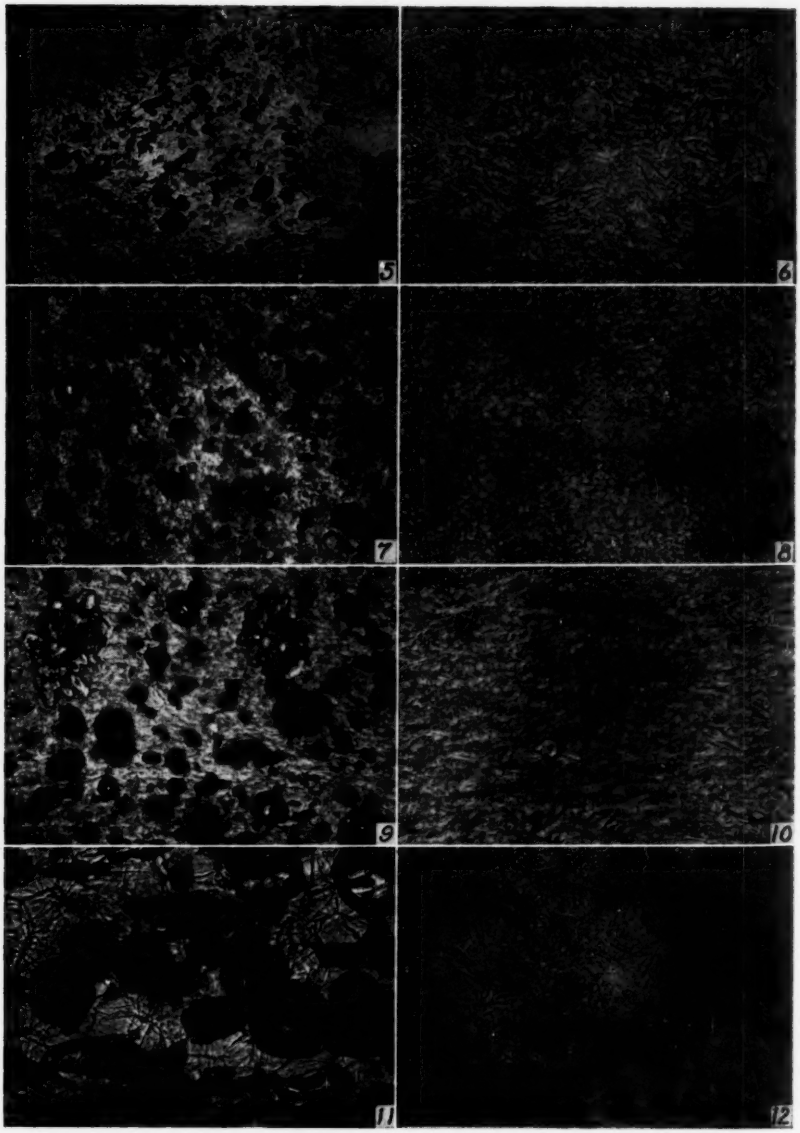
When the tissue starts to dry down, the cells lose turgor, and the walls become more or less flaccid. The surface, if teased with a needle, has a sticky consistency and the mass of tissue flattens down on the cover glass. As loss of moisture proceeds, the surface dries and the walls become rigid. As this drying of the walls continues into the tissue, sap previously released from cells evaporates down to a syrup coating the walls and the intercellular air spaces become larger and larger. Finally, the completely dry tissue appears as a thin layer of plastic material glued fast to the cover glass, with the outlines of cells still visible but with the walls white or dull gray instead of moist and glistening as in the fresh tissue. Trapped within the intercellular spaces are many bubbles, larger than they were originally and mostly spherical or nearly so.

Thin sections cut from dehydrated fruits look much the same as these thin sections dried under the microscope; but they represent tissue shrunk in three dimensions, whereas tissue dried on a glass surface has contracted in only one, being cemented to the glass by the drying sap so that horizontal contraction is prevented.

Thin sections of apricot, peach, and pear (Figs. 5, 7, and 9), respectively, cut from fruits dehydrated without blanching, show that the cells have collapsed and much air has been entrapped in intercellular spaces. In the upper left (Fig. 5) is a small portion of a vascular bundle, and on the right margin is a sap-filled cavity caused by the extreme shrinkage that occurs. Two stone-cell groups are visible (Fig. 9). In a section of apple tissue dried on a cover slip under the microscope (Fig. 11) obviously vertical contraction alone has taken place, for the outlines of cells can be seen in their original dimensions. In sections of cabbage petiole, carrot root, and sweet potato (Figs. 13, 15, and 17), respectively, dried on the slide under the microscope, intercellular air trapped in the tissues is obvious in all three. Starch grains are also apparent in the sweet-potato tissue.

The white potato among vegetables is analogous to the prune among fruits in that the cells are relatively large and there are few air-filled intercellular spaces. The whiteness of the fresh-cut tissue is caused by reflection of light from the surfaces of the many starch grains present rather than from bubbles. A thin section of potato dried on a cover slip under the microscope (Fig. 19) shows intercellular air as well as the outlines of cells completely obscured by the many starch grains. Drying was so rapid that little gelation of starch occurred though the temperature was above that of gelation for potato starch.

So far the drying only of unblanched tissues has been described. Drying of blanched tissues is very different. As mentioned in the previous section, blanching rids the tissue of intercellular air. Drying of such tissue shows simply a graying of the exposed walls as they lose their surface film of moisture, a gradual shrinking of the tissue, and finally a change from a clear, translucent appearance to a rather dull one as the section dries down hard on the glass surface. In such a section, again, the original outlines of the cells can be seen; but no bubbles are present



FIGS. 5 to 10. Thin hand sections of dehydrated fruits mounted in glycerine. ×84.
FIGS. 11 and 12. Thin hand sections of apple dried on slide under microscope. ×84.

Fig. 5. Royal apricot, unblanched, in which air bubbles are prominent throughout the tissue. On the right center is a cavity formed by the parting of the tissue caused by shrinkage during drying. On the upper left is a short piece of a vascular bundle. Fig. 6. Blanched, dehydrated Royal apricot. Folding of the cell walls and compression of the tissue is prominent. Fig. 7. Unblanched Muir peach with many air bubbles present in the tissue. Fig. 8. Blanched Muir peach in which the tissue is quite uniform in texture. Fig. 9. Unblanched Bartlett pear with two stone-cell groups visible. Fig. 10. Blanched Bartlett pear with stone cells in the center. Fig. 11. Unblanched section of Newtown Pippin apple dried on the slide. The very large and numerous air bubbles give dried apples their intense white appearance. Fig. 12. Blanched Newtown apple in which bubbles are completely absent.

and the sap in the cells in the form of a hardened glassy syrup is fairly translucent. Sections of dried fruits have similar properties except for the folding of the cell walls as the drying fruit piece contracts in all three dimensions.

Sections of blanched dehydrated apricot, peach, and pear (Figs. 6, 8, and 10), respectively, show the accordion pleat-like folding of the walls characteristic of such tissue. The dense lines, especially evident in Fig. 6, represent the cell walls; a stone-cell group is present in Fig. 10.

A section of apple tissue dried on the slide after blanching (Fig. 12) shows the approximate outlines of the cells though the superposition of several cells makes the outline somewhat confused. Sections of cabbage petiole, carrot root, and sweet potato (Figs. 14, 16, and 18), respectively, blanched and dried under the microscope, all show the cell outlines; the aggregation of pigment bodies previously mentioned may be seen (Fig. 18). These aggregations contained carotinoid pigments and showed as irregular yellow dots in the killed, dried cells. Starch in the sweet-potato section has been gelled and is scarcely visible.

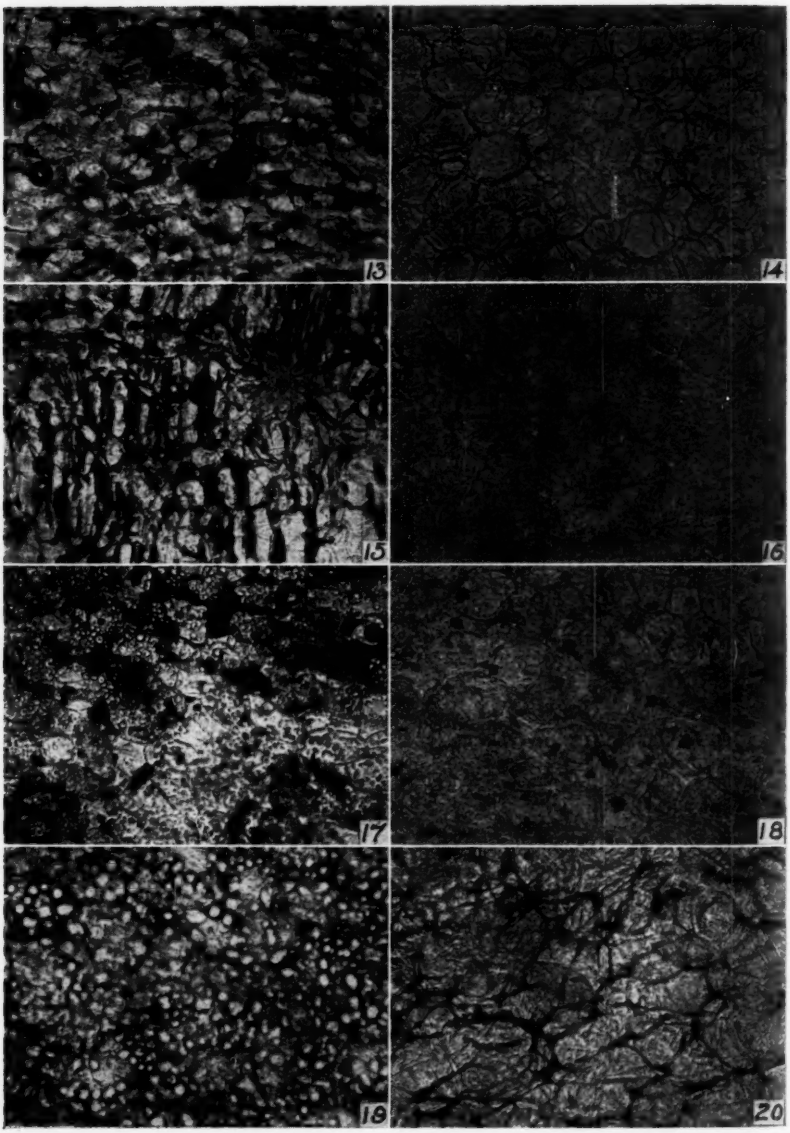
A section of white potato blanched and dried under the microscope is shown (Fig. 20). As the temperature of this section reached the gelation point, the starch grains could be seen to expand rapidly with a bursting action as if they were retained within membranes that broke. They soon filled the cells completely with a reticulate, gray, translucent mass that almost obscured the cell outlines. Fine, air-filled intercellular spaces, on the other hand, failed to lose their air and therefore appear as black lines of varying width in the photomicrograph. Had the section been heated longer, the air would have been displaced by water vapor and finally lost. More time is required in potato, however, because the moisture normally lost from cells as they are killed by blanching is absorbed by the gelling starch, leaving the cells relatively free of fluid water.

DISCUSSION

As indicated earlier, when plant tissues dry, surface forces are set up that exert a definite influence upon the various phases of the system. Upon drying of a rigid structure, such as a brick or a stiff sponge, water is lost first from the large spaces, then from smaller and smaller ones; and finally the surface film that tends to wet the entire internal surface is lost. In a colloidal material, such as clay soil or the flesh of a prune, considerable heat energy must be applied to remove this last film in the presence of normal moist air.

As fruit tissue dries, the bubbles in the intercellular space system enlarge; the same type of drying proceeds except that sap released from cells tends to retard the process and, as this sap is lost by evaporation, the tissue shrinks.

When the intercellular air has been completely replaced by water or sap from the cells, the situation is different. In the drying of such tissue, surface forces tend to exert a strong compression. Only when rigid structures are present are bubbles formed. If good-quality fruit is steam-blanched until the cell walls become soft and plastic, it will dry down without bubble formation. If a few small bubbles chance to be left after



FIGS. 13 to 20. Sections of dehydrated vegetable tissues dried on the slide and mounted in glycerine. ×84.

Fig. 13. Unblanched section of cabbage petiole. The large round bubbles were formed under the cover slip after the glycerine was applied. They are composed of air that was displaced from intercellular spaces in the tissue by the glycerine because of capillarity. Fig. 14. Blanched cabbage petiole. Fig. 15. Unblanched carrot tissue in which air is particularly abundant in the ray tissues of the carrot root. Fig. 16. Blanched carrot tissue. Fig. 17. Unblanched sweet potato with intercellular air present in large, irregularly shaped, intercellular spaces. Starch is abundant in the cells. Fig. 18. Blanched sweet potato in which starch has been gelled and pigment bodies have aggregated into clumps. Fig. 19. Unblanched Netted Gem potato tissue dried on the slide. Intercellular air is almost completely obscured by the many starch grains, and cell outlines are barely visible in places. Fig. 20. Blanched potato in which the starch is completely gelled, forming a gray reticulate mass in each cell. Intercellular air is still present in minute passages between the cells.

blanching, these will slowly collapse and disappear under the compressional force of surface tension that presses the gases into solution.

Fruit in the sulfur house may become completely translucent by replacement of intercellular air by sap. When dried in the dehydrator, however, such fruit becomes opaque and full of bubbles again. The cell walls have not become plastic enough to give, and they form rigid intercellular compartments within which bubbles are formed under the tension developed as liquid is withdrawn. During gradual sun-drying, however, the heating plus the slowness of the drying allows for bending of the walls, and bubbles are not reformed.

Blanched fruit, on the other hand, may be rapidly dried without bubble formation. Here the high temperature has rendered the walls plastic, and they give under the compressional forces set up.

The discussion thus far has emphasized the effect of blanching upon the appearance of the product. If appearance alone were improved, the process could scarcely be justified, for the public could be re-educated to use a different-looking product. Other advantages, however, accrue, according to Crafts (1944):

1. Intercellular air acts as an insulator restricting the inward movement of heat and outward movement of moisture. Experience has proved that blanched products dry more quickly than unblanched.

2. Air trapped in drying tissues contains oxygen that may react with ascorbic acid, carotene, and other easily oxidized constituents. As Mrak, Phaff, Fisher, and Mackinney (1943) have shown, blanched fruits retain their vitamin C content longer than unblanched. Cruess and Mackinney (1943) report similar results for both carotene and vitamin C in vegetables.

3. Though the change in appearance upon blanching may not be of primary importance, it is an index of complete penetration of heat. Such penetration brings about at least two beneficial reactions: first, it inactivates certain enzymes; second, it softens cell walls and improves the texture of the cooked product—Simpson and Halliday (1941). This softening also, as explained above, prevents the reformation of bubbles that would contain oxygen to react with the tissues.

The various chemical indicators now used to determine the activity of enzymes in tissues after blanching are doubtless of value. If, however, the altered appearance could be accurately correlated with enzyme inactivation, as indicated by the photographs in Fig. 1 of the report by Mrak, Phaff, Fisher, and Mackinney (1943), then this change in appearance brought about by replacement of intercellular air might prove a sufficient index of adequate blanching.

The studies reported here serve well to illustrate how the techniques of the research laboratory may be applied to practical problems in preservation of food products.

SUMMARY

Sections of the fleshy tissue of fruits and vegetables were observed under the microscope during steam-blanching and dehydration.

Fleshy parenchyma of apricot, peach, pear, and apple and of cabbage, carrot, and sweet potato is characterized by intercellular space filled with gas. If such tissue is dried without blanching, intercellular air is trapped among the cells.

Steam-blanching rids the tissues of intercellular air and softens the cell walls. Steam-blanched tissue dries down in a bright, translucent condition, relatively free of intercellular air.

Intercellular air restricts the free movement of moisture and heat through the tissue and hinders dehydration. Oxygen in the intercellular air in tissues may react with vitamins and other labile materials, lowering the quality of the product during storage. Freedom from intercellular air may serve as an index of thorough blanching. Thus translucency of the tissue is a rapid, indirect measure of the inactivation of enzymes and softening of the cell walls.

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ASCORBIC ACID CONTENT OF SMALL FRUITS IN RELATION TO GENETIC AND ENVIRONMENTAL FACTORS[1]

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The breeding of improved varieties of small fruits for fresh market, canning, and other purposes has been in progress at the Oregon Experiment Station for more than 25 years. Since the ascorbic acid content is one of the principal nutritional values of these fruits, it seemed desirable to consider breeding as a means of obtaining varieties uniformly high in vitamin C potency. No information was available regarding the ascorbic acid content of the small fruits grown in Oregon, but data from other regions have shown that some varieties are richer sources of this vitamin than others. In 10 varieties of red raspberries grown in the state of Washington, Todhunter and Robbins (1941) found a range of 13 to 35 mg. of ascorbic acid per 100 grams. Varieties of small fruits in New York, according to Kirk and Tressler (1941); North Carolina, Lineberry and Burkhart (1942); and South Carolina, Satterfield and Yarbrough (1940) also varied considerably in ascorbic acid content. Burkhart and Lineberry (1942) found also that the vitamin C content varied according to the size and ripeness of the fruit as well as to light intensity and the location where the plants were grown.

In this investigation the ascorbic acid content of small-fruit varieties of commercial importance in Oregon has been determined. Data are included also on the vitamin C potency of selections developed by breeding, which are being tested for possible introduction as commercial varieties.

EXPERIMENTAL PROCEDURE

Method of Analysis: Twenty-five grams of berries were transferred to a Waring blender and extracted with 300 ml. of one-per cent metaphosphoric acid, Loeffler and Ponting (1942). After filtering or centrifuging, five ml. of the extracts were pipetted into 50-ml. volumetric flasks and made to volume with sodium citrate-metaphosphoric acid buffer solution at pH 3.6. Ascorbic acid was then determined by the method of Morell (1941), adapted for use with a Klett-Summerson photometer. To determine the accuracy of this method, aliquots of the metaphosphoric acid extracts of strawberries and gooseberries were determined according to the procedure specified and also by titration with 2,6-dichlorobenzenone-indophenol. Values of 58.9 and 59.4 mg. for gooseberry, and 50.5 and 50.7 mg. per 100 grams for strawberry were obtained, respectively, by the two methods.

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Source of Material and Method of Sampling: The samples were obtained from experimental plots located near Corvallis. The strawberries were grown on three different plots. Plot 1 was a randomized block containing two commercial varieties and 20 selections (selected seedlings of various crosses on trial for possible introduction as commercial varieties). The second plot consisted of two named varieties and three selections, replicates of which were grown in either Plots 1 or 3 or both. Plot 3 contained one variety and 13 selections, a portion of these being replicates of those grown elsewhere. Plot 1 was located on Chehalis silty clay loam soil which had received an application of manure in the spring and was irrigated once during the harvest season. Plot 2 was located on the same type of soil but no manure had been applied. Plot 3 was located approximately one mile from the others on Chehalis fine sandy loam soil. No fertilizer or irrigation was applied to this plot. The raspberry and blackberry varieties and selections were grown on closely adjacent plots having the same type of soil.

Samples were collected in the morning at weekly intervals throughout the harvest season. Only fully ripe berries were used. The strawberries and blackberries were sampled by cutting a transverse section through the center of the berry, using 25 to 30 sections for a 25-gram sample. Raspberries were sampled by slicing through the longitudinal axis. The samples were covered immediately after weighing with 300 ml. of one-per cent metaphosphoric acid. Preliminary tests showed that this method of sampling gave results comparable to those obtained with whole berries. Berries which were not analyzed within six hours were placed in Pliofilm bags and stored at 5.6°C.(42°F.) for periods not exceeding 24 hours. Preliminary tests showed that samples could be kept under these conditions for at least 72 hours without significant change in weight or ascorbic acid content.

RESULTS AND DISCUSSION

Variability Among Varieties and Selections: The ascorbic acid content of 30 strawberry, 27 red raspberry, and 20 blackberry and dewberry varieties and selections are shown (Tables 1, 2, and 3). The mean ascorbic acid content of the strawberry varieties and selections ranged from 52.6 to 107.1 mg. per 100 grams of fresh fruit. The range in ascorbic acid content of raspberries was 17.7 to 37.0 mg., and in blackberries and dewberries 12.1 to 27.5 mg. Although the values for each variety and selection varied during the season, they mostly tended to remain within either a low, a medium, or a high range. Thus, ascorbic acid content of the highest ranking strawberry selection ranged from 120 to 90 mg., while that of the lowest ranged from 58 to 48 mg. It is evident from these data that the genetic constitution of a variety has a determining influence on its ascorbic acid content.

The strawberry selections and recently introduced varieties mostly have been derived through several generations of breeding from two or more varieties (Table 4). Those utilized most often in the original crosses were Marshall, Ettersburg 121, and Clark. The native wild species, *Fragaria chiloensis* and *F. ovalis,* and certain varieties originated by the United States Department of Agriculture also were crossed with these Western

varieties and species. Later, selections from progeny of the earlier crosses were back-crossed, and crosses between selections have been made also. Insufficient information is available regarding the ascorbic acid content of the varieties and selections used in breeding to determine how ascorbic acid potency has been transmitted. The data suggest, however, that certain varieties have been more influential than others in obtaining progeny with high ascorbic acid content. Thus, Marshall appears in nine of the 12 highest but in only three of the 12 lowest rating varieties and selections. Furthermore, the average percentage of Marshall is greater in the 12

TABLE 1

Ascorbic Acid Content of Strawberry Varieties and Selections

Variety or selection	Plot	Number of samples	Ascorbic acid in fresh fruit	
			Range	Mean [1]
			mg./ 100 gm.	*mg./ 100 gm.*
Ore. 1541	3	4	120-90	107.1
Ore. 1541	1	3	106-98	103.2
Ore. 1553	1	5	105-91	98.4
Ore. 1629	2	4	108-86	97.3
Ore. 1629	1	6	105-89	96.3
Ore. 1807	3	5	105-84	95.9
Ore. 1629	3	3	104-90	95.5
Ore. 1809	1	3	108-79	94.3
Ore. 1564	1	5	103-85	92.9
Ore. 1429	3	4	105-79	92.3
Ore. 1797	3	4	95-85	91.9
Ore. 1429	2	4	98-85	90.5
Ore. 1798	1	4	94-87	90.3
Corvallis	1	4	94-78	86.1
Corvallis	2	4	88-78	82.8
Ore. 1545	1	4	95-74	82.2
Ore. 1582	3	4	90-70	82.1
Ore. 1429	1	7	86-74	80.2
Ore. 1545	2	4	90-63	79.8
Marshall	1	6	91-66	78.4
Ore. 1582	1	6	88-72	78.3
Marshall	2	3	80-74	77.3
U. S. 3328	3	6	82-66	76.7
Ore. 1420	1	5	81-70	76.1
Ore. 1087	1	6	84-67	75.6
Maytime	3	5	81-62	72.2
Ore. 1802	3	5	78-63	71.9
Ore. 1816	3	4	76-66	70.9
Ore. 1491	1	6	78-63	70.3
Ore. 1114	1	6	76-61	68.7
Ore. 1803	3	5	78-58	68.5
Brightmore	1	7	73-63	68.0
Ore. 1622	1	6	82-63	66.9
Ore. 1639	1	4	81-59	66.1
Ore. 1191	1	4	69-59	64.5
U. S. 3378	3	5	68-54	64.4
Ore. 1090	1	5	65-55	60.5
Ore. 1082	3	4	62-49	55.3
Ore. 1581	3	6	58-48	52.6

[1] Significant difference at five-per cent level = 9.8 mg.

highest than in the 12 lowest rating selections. The varieties, Clark, Progressive, and *F. chiloensis*, also appear more often in the selections having high ascorbic acid content.

In red raspberries the majority of the selections (Table 2) originated from crosses of Lloyd George with other varieties. Fruit of Lloyd George was not available during the current season, but data from the states of

TABLE 2

Ascorbic Acid Content of Red Raspberry Varieties and Selections

Variety or selection	Parentage	Number of samples	Ascorbic acid in fresh fruit	
			Range	Mean [1]
			mg./ 100 gm.	*mg./ 100 gm.*
Ore. 345	Lloyd George x Chief	3	43.34	37.5
Newman	Unknown	5	41-32	37.0
Ohta	Native sp. x Minnetonka	5	38-35	36.1
N. Y. 13641	L. George x Newburgh	5	40-34	35.7
Ore. 550	Viking x L. George	5	36-33	34.6
Chief	Latham seedling	4	37-33	34.6
Ore. 273	Ranere x L. George	6	37-33	34.2
Rote Riesen	Unknown	5	36-29	32.2
Willamette	Newburgh x L. George	6	34-27	31.0
Ranere	Unknown	5	34-27	29.2
Ore. 336	Cuthbert x L. George	4	32-26	28.1
Ore. 402	Viking x L. George	3	33-25	27.9
Ore. 549	Viking x L. George	3	27-25	26.3
Ore. 663	L. George x Cuthbert	6	30-21	26.0
Ore. 349	Viking x L. George	4	29-24	26.0
Ore 564	Viking x L. George	3	27-23	25.7
Taylor	Newman x L. George	5	29-21	24.7
Ore. 410	Newburgh x L. George	4	26-23	23.9
Ore. 368	Cuthbert x L. George	5	29-21	23.8
Cuthbert	Unknown	5	26-21	23.2
Washington	Cuthbert x L. George	5	26-20	22.7
Ore. 331	Viking x L. George	4	25-21	22.5
Ore. 140	Cuthbert x L. George	3	23-21	21.4
Ore. 169	Cuthbert x L. George	4	24-18	21.0
Ore. 575	L. George x Cuthbert	6	22-19	19.3
Tahoma	Latham x L. George	7	23-16	19.1
Ore. 563	L. George x Cuthbert	4	21-16	17.7

[1] Significant difference at five-per cent level = 3.3 mg.

Washington and New York indicate that it is comparatively low in ascorbic acid content (13 to 17 mg.). The selections rating high were found to be from crosses of Lloyd George with Chief, Ranere, and Newburgh. The varieties, Chief and Ranere, were high in ascorbic acid content, while Newburgh has been reported by Kirk and Tressler (1940) to be of high potency in New York but of lower potency in North Carolina by Lineberry and Burkhart (1942). The majority of the selections low in vitamin C content are from crosses of Lloyd George with Cuthbert, a low-ranking variety. However, certain exceptions can be noted. The variety, Taylor, originating from a cross of Newman x Lloyd George, had a mean of 24.7 mg., which is higher than one parent but considerably lower than the other.

Progeny of Viking x Lloyd George were found to be variable in ascorbic acid content.

In blackberries and dewberries the two highest rating selections originated from crosses between Zeilinski and Logan, both of which rank high in this group of small fruits. The Pacific blackberry, which also originated from this cross, was intermediate in ascorbic acid content. The selections, Ore. 609 and Ore. 676, both low in ascorbic acid, originated from a cross of Black Logan x Youngberry, which varieties are low and intermediate, respectively, in potency. Selections having Himalaya as a parent were variable in their ascorbic acid content.

TABLE 3

Ascorbic Acid Content of Blackberry and Dewberry Varieties and Selections

Variety or selection	Parentage	Number of samples	Ascorbic acid in fresh fruit	
			Range	Mean [1]
			mg./ 100 gm.	*mg./ 100 gm.*
Ore. 616	Zeilinski x Logan	4	34-24	27.5
Cascade	Zeilinski x Logan	6	36-19	26.6
U. S. 28	Himalaya x Unknown	4	34-20	26.0
Logan	Seedling of Auginbaugh	5	34-17	25.8
Zeilinski	Unknown	4	28-22	25.4
Ore. 731	Santiam x Himalaya	6	31-21	25.2
Ashton Cross	Unknown	3	26-23	24.2
Young	Phenomenal x Mayes	3	29-22	23.8
Pacific	Zeilinski x Logan	5	27-20	22.6
Himalaya	Unknown	3	27-19	21.7
Ore. 743	Pacific x Boysen	3	25-18	21.4
Boysen	Unknown	4	25-14	20.5
Nectar	Seedling of Young	3	24-16	20.5
Lucretia	Unknown	5	22-13	17.7
U. S. 51	Himalaya x Evergreen	3	19-14	16.4
Ore. 609	Black Logan x Young	5	23-12	16.2
Ore. 266	Himalaya x Texas	4	18-13	16.1
Ore. 676	Black Logan x Young	4	20-13	16.0
Black Logan	Unknown	4	18-11	14.0
Texas (Eldorado)	Unknown	3	12-	12.1

[1] Significant difference at five-per cent level = 3.7 mg.

Hereditary differences in the ascorbic acid concentration of strawberry varieties and selections do not appear to be associated with any visible plant or fruit characters. Burkhart and Lineberry (1942) have shown that size of berry and exposure to light influence ascorbic acid content, and these observations have been confirmed in work preliminary to the present investigations. The differences owing to these factors, however, were not great enough to account for the extreme variability found within the various varieties and selections. With respect to light, some of the highest ranking selections bore the berries densely shaded by the leaves; while others, consistently low in ascorbic acid, bore the fruit on long upright stems, fully exposed to the sun. Color of ripe fruit, vigor of plant, and amount of leaf surface apparently have little influence. Fruit from strawberry plants affected with "red stele" disease, which greatly reduces

TABLE 4

Derivation of Strawberry Varieties and Selections

Variety or selection	Per cent of each variety used in breeding											
	Aberdeen	Clark	Ettersburg 121	Gold Dollar	Howard 17	Marshall	Missionary	Progressive	Redheart	Royal Sovereign	*F. chiloensis*	*F. ovalis*
Ore. 1541	.....	.....	12.5	.....	12.5	25.0	12.5	12.5	.....	.....	25.0	.....
Ore. 1553	.....	25.0	12.5	.....	12.5	.....	.....	12.5	.....	12.5	25.0	.....
Ore. 1629	.....	.....	25.0	.....	25.0	25.0	.....	.....	.....	25.0	.....	.....
Ore. 1807	.....	.....	12.5	.....	12.5	50.0	12.5	.....	.....	.....	12.5	.....
Ore. 1809	.....	.....	12.5	.....	12.5	50.0	12.5	.....	.....	.....	12.5	.....
Ore. 1564	.....	.....	12.5	.....	25.0	37.5	12.5	.....	.....	12.5	.....	.....
Ore. 1429	.....	25.0	.....	.....	25.0	12.5	25.0	.....	.....	.....	12.5	.....
Ore. 1797	.....	.....	50.0	.....	.....	25.0	.....	.....	25.0	.....	.....	.....
Ore. 1798	.....	25.0	.....	.....	25.0	12.5	12.5	.....	.....	12.5	12.5	.....
Corvallis	.....	.....	50.0	.....	.....	50.0	.....	.....	.....	.....	.....	.....
Ore. 1545	.....	25.0	12.5	.....	12.5	.....	.....	.....	.....	12.5	25.0	.....
Ore. 1582	.....	25.0	25.0	.....	12.5	.....	12.5	12.5	.....	.....	.....	25.0
Marshall	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
U. S. 3328	50.0	.....	.....	.....	25.0	.....	.....	.....	.....	25.0	.....	.....
Ore. 1420	.....	.....	25.0	25.0	12.5	25.0	.....	.....	.....	12.5	.....	.....
Ore. 1087	.....	.....	.....	.....	25.0	25.0	25.0	.....	.....	.....	25.0	.....
Maytime	.....	.....	.....	.....	25.0	.....	50.0	.....	.....	25.0	.....	.....
Ore. 1802	.....	.....	25.0	.....	25.0	.....	12.5	.....	.....	12.5	25.0	.....
Ore. 1816	.....	12.5	37.5	.....	18.75	.....	18.75	.....	.....	.....	.....	12.5
Ore. 1491	.....	.....	12.5	.....	25.0	12.5	.....	12.5	.....	25.0	12.5	.....
Ore. 1114	.....	.....	25.0	.....	25.0	25.0	25.0	.....	.....	.....	.....	.....
Ore. 1803	.....	25.0	25.0	.....	12.5	.....	12.5	.....	.....	.....	.....	25.0
Brightmore	.....	.....	25.0	.....	25.0	25.0	25.0	.....	.....	.....	.....	.....
Ore. 1622	.....	.....	25.0	.....	37.5	.....	12.5	.....	.....	25.0	.....	.....
Ore. 1639	.....	.....	25.0	.....	37.5	.....	.....	.....	.....	37.5	.....	.....
Ore. 1191	.....	.....	50.0	.....	25.0	.....	25.0	.....	.....	.....	.....	.....
U. S. 3378	50.0	.....	.....	.....	25.0	.....	.....	.....	.....	25.0	.....	.....
Ore. 1090	.....	.....	25.0	.....	25.0	.....	25.0	.....	.....	.....	25.0	.....
Ore. 1082	.....	.....	25.0	.....	25.0	.....	25.0	.....	.....	.....	25.0	.....
Ore. 1581	.....	25.0	25.0	.....	12.5	.....	12.5	.....	.....	.....	.....	25.0

vigor, were found to contain as much ascorbic acid as fruit from unaffected, vigorous plants of the same variety.

The leaves of strawberry plants were found to be high in ascorbic acid, ranging from 319 to 435 mg. per 100 grams fresh weight. The concentration of ascorbic acid in the leaves of raspberry selections and varieties ranged from 182 to 245 mg. No correlation, however, was found between the amounts of the vitamin in the leaves and in the fruit. These data indicate that some varieties may have greater ability than others for translocation of ascorbic acid (or a precursor) from the levels to the fruit.

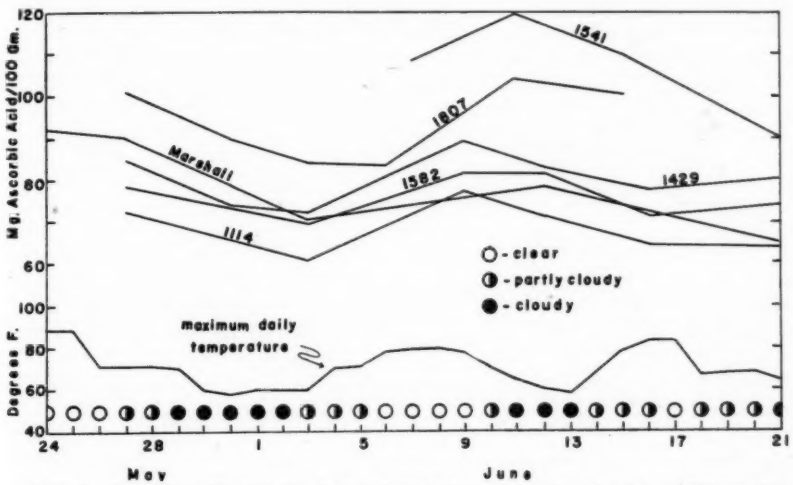

FIG. 1. Ascorbic acid content of strawberries in relation to climatic conditions during the harvest season.

Seasonal Variability Within Individual Varieties and Selections: The ascorbic acid content of individual varieties and selections fluctuated more or less during the harvest season (Tables 1, 2, and 3). In the case of strawberries, these fluctuations can be accounted for in part by differences in climatic conditions at various periods during the season. Strawberries picked during periods of prevailing sunshine generally tended to be higher in ascorbic acid than those picked during periods of cloudy weather (Fig. 1). Evidence for the influence of light was obtained in the following experiment: Two plots of Marshall plants were covered with frames constructed with single and double layers of unbleached muslin. On a third plot the clusters of berries only were covered with muslin bags, while on the fourth plot the leaves and fruits were left uncovered. After a period of 21 days, ripe berries from each plot were analyzed for ascorbic acid. The results show (Table 5) that berries grown and ripened under reduced light intensity contained less ascorbic acid than those ripened on plants fully exposed to the light. Shading the entire plants resulted in a much greater reduction in ascorbic acid than did shading of the berries only. These data indicate that while the berries are able to synthesize a

portion of the vitamin C which they contain, the larger amount is synthesized in the leaves and translocated to the fruit.

The ascorbic acid content of red raspberries was found to be affected also by light intensity. Berries of two selections grown and ripened under black sateen bags contained 23 and 15.1 mg. of ascorbic acid, while fruit of the same selections grown and ripened in the open contained 30.5 and 18.6 mg., respectively. No significant differences, however, were found between the ascorbic acid content of berries collected from the north and south sides of the rows.

TABLE 5

Effect of Shading on Ascorbic Acid Content of Strawberries

Treatment	Ascorbic acid
	mg./100 gm.
Unshaded	80.2
Berries only shaded	70.9
Plants shaded 1X	64.4
Plants shaded 2X	47.6

Seasonal fluctuations in the ascorbic acid content of raspberries ranged from two to nine mg. (mean, 5.6 mg.) for the 27 varieties and selections tested. This comparatively low variation may be accounted for partly by uniformity in the amount of clear weather prevailing during the harvest season of these fruits. Some selections of blackberries and dewberries showed little fluctuation from one picking to another, while others varied considerably. In some cases there was a noticeable trend towards a decrease in ascorbic acid content as the season advanced. In the Cascade blackberry the ascorbic acid contents of six samples collected from the beginning to the end of the season were 35, 36, 24, 24, 20, and 19 mg., and for Logan 34, 28, 28, 24, and 26 mg. Several other varieties and selections showed similar consistent decreases.

TABLE 6

Ascorbic Acid Content of Strawberries Grown on Three Different Plots

Variety or selection	Ascorbic acid in fresh fruit		
	Plot 1	Plot 2	Plot 3
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*
Corvallis	82.8	.....	82.3
Marshall	78.4	77.3	.....
Ore. 1429	80.2	90.5	92.3
Ore. 1541	103.2	.....	107.1
Ore. 1545	82.2	79.8	.....
Ore. 1582	78.3	.....	82.1
Ore. 1629	96.3	97.3	95.5

The ascorbic acid content of the same varieties and selections of strawberries grown on different plots (Table 6) showed little variation, with one exception. Burkhart and Lineberry (1942) found that values for Klondike strawberries grown in six fields in North Carolina ranged from 36.3 to 52.2

mg. Variations in soil and other environmental factors may have been greater than those encountered in the present experiments. Moreover, some varieties may be more responsive to change in environments than others.

SUMMARY

A study has been made of the variability in the ascorbic acid content of small-fruit varieties and selections grown in Oregon. The range in milligrams of ascorbic acid per 100 grams of fresh fruit was 52.6 to 107.1 for strawberry, 17.7 to 37.5 for red raspberry, and 12.1 to 27.5 for blackberry and dewberry varieties and selections. Certain of the selections originated by breeding were found to contain more ascorbic acid than the commercially grown varieties.

High ascorbic acid potency in the strawberry selections appeared to have been influenced more by Marshall, Clark, Progressive, and *F. chiloensis* than by other varieties or species used in breeding. In red raspberries the selections highest in ascorbic acid content originated from crosses of Lloyd George (low potency) with Chief, Newburgh, or Ranere (intermediate to high potency). The highest ranking blackberry selections were progeny of a cross between Zeilinski and Logan. Both of these varieties are high in ascorbic acid content.

The ascorbic acid values for individual varieties and selections varied during the harvest season. These variations appear to be related to differences in the climatic conditions prevailing during the period of growth and ripening. In some varieties of blackberries there was a decrease in ascorbic acid content as the season advanced. Strawberries grown on several different plots, with one exception, showed close agreement in ascorbic acid content.

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RATIO OF SOLUBLE SUGARS, PECTIC MATERIALS, AND HEMICELLULOSES TO NITROGEN-FREE EXTRACT OF SOME COMMON VEGETABLES[1]

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How much soluble sugars, pectic compounds, and hemicelluloses are furnished by our common edible vegetables? The numerical value of these constituents is usually masked by the nitrogen-free extract which includes all the carbohydrates except cellulose, part of the cellulosan group, some of the lignin, and other constituents. It follows therefore that the procurement of information on the content of the constituents found in this fraction involves special investigations—Williams, Wicks, Bierman, and Olmsted (1940); Maynard (1940); Heller and Well (1940)—and the data are consequently incomplete. They are present in too-great quantities and play too great a role in the economy of the life of the plant to be considered as anything but definite entities. The content of this paper indicates the extent to which soluble sugars, pectic materials, and hemicelluloses may occur in some of our common vegetables, and their quantative relationship to the nitrogen-free extract. The data on the hemicelluloses are different from those usually reported in that the values were obtained directly by isolating the material.

Comments concerning the soluble sugars are unnecessary since the emphasis is on the percentage content rather than on qualitative differences. Pectic materials include free pectin, protopectin, and pectates. No attempt was made to differentiate between these fractions; hence the values reported include all fractions determined as calcium pectate. According to a differentiation by Hawley and Norman (1932), hemicelluloses may be classed as polyoses, polyuronides, or cellulosans. In this paper the term hemicellulose includes the above fractions, thereby including all polysaccharide material associated with the cell walls which is soluble in dilute alkali, but excluding pectic and water-soluble materials.

EXPERIMENTAL PROCEDURE

Several pounds of each fresh vegetable[2] were obtained, at as near the same time of day as possible and at comparable stages of maturity, direct from the fields consisting of Merrimac fine sandy loam soil. The material was cut up and dried in a forced-draft, hot-air oven operating at about 60°C.(140°F.). The dried samples were then finely ground in a Wiley mill.

Soluble sugars were extracted with hot 80-per cent ethyl alcohol. The aqueous solutions of the sugars were clarified, then hydrolyzed with hydrochloric acid at room temperature. The reducing power was determined by the Lane-Eynon method (1940).

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[1] Contribution 507 of the Massachusetts Agricultural Experiment Station.

[2] The author is indebted to Mr. W. H. Lachman, of the Department of Olericulture, for samples used in this investigation.

The determination of pectic substances was preceded by an extraction with hot 80-per cent ethyl alcohol until the extract was substantially free from pigment. The residue was then dried and extracted with 0.6 per cent dibasic ammonium citrate at 80°C.(176°F.) for a total of about 45 hours. Calcium pectate was determined in these extracts according to the procedure of Conrad (1926).

Hemicelluloses were isolated and determined gravimetrically, according to Buston (1934). Previous to the actual extraction of hemicelluloses, pigments and pectic substances were removed. The total weight of hemicelluloses was corrected for nitrogen and ash.

Original moisture was determined by the method of Bidwell and Sterling (1940), using 25-ml. receiving tubes.

TABLE 1

Content of Nitrogen-Free Extract, Total Sugars, Hemicelluloses, and Pectic Compounds on a Dry-Matter Basis in Some Common Vegetables

Vegetable	Nitrogen-free extract[1] (A)	Soluble sugars (B)	Pectic materials (C)	Hemicelluloses (D)	Ratio	
					B/A	C+D / A
	pct.	*pct.*	*pct.*	*pct.*		
Asparagus, *Asparagus officinalis* L.	42.90	14.67	Trace	4.70	.342	.110
Cabbage, *Brassica oleracea* L.	57.73	33.09	4.57	5.20	.573	.169
Carrot, *Daucus carota* L.	77.47	51.40	7.41	6.72	.663	.182
Celery, *Apium graveolens* L.	50.34	9.67	7.38	5.38	.192	.253
Dandelion, *Taraxacum officinale* W.	48.18	7.49	7.81	6.12	.155	.289
Eggplant, *Solanum melongena* L.	60.47	29.35	11.03	7.74	.485	.310
Head lettuce, *Lactuca sativa* L.	53.25	20.60	4.00	3.46	.387	.140
Kale, *Brassica oleracea* L., var. *acephala*	.....	9.67	4.55	7.60	.....	.....
Peppers, *Capsicum frutescens* L.	64.73	37.46	7.68	4.30	.579	.185
Radish, *Raphanus sativus* L.	54.34	19.05	7.78	4.38	.351	.224
Spinach, *Spinacia oleracea* L.	33.07	4.06	11.58	5.70	.123	.523
String beans, *Phaseolus vulgaris* L.	57.83	24.35	5.02	9.06	.421	.243
Squash, *Cucurbita maxima* D.	86.45	48.76	3.32	12.00	.564	.177
Turnip, *Brassica rapa* L.	73.94	49.10	5.07	4.16	.664	.125

[1] Holland and Ritchie (1941).

The data presented indicate that the percentage content of soluble sugars may range from about four to 51 per cent of the dry matter (Table 1). These values represent about 12 to 66 per cent of the nitrogen-free extract, depending upon the type of plant.

The pectic materials and hemicelluloses, collectively, may constitute about five to 19 per cent of the dry matter which represents about 11 to 52 per cent of the nitrogen-free extract. Although these substances have a variable and questionable nutritive value, the evaluation of their full significance to the human body should be reserved until further work has been done on their physical and chemical nature.

From the above data it is evident that the amount of soluble sugars, pectic compounds, and hemicelluloses varies with the species, and that the numerical value of the nitrogen-free extract gives little information regarding the relative amounts of these fractions.

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RIBOFLAVIN CONTENT OF FRESH AND PROCESSED SWISS CHARD[1]

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This is the third paper of a series [Porter, Schlaphoff, Wharton, Briant, and Beltz (1944); Porter, Wharton, and Beltz (1944)] concerned with the effect of cooking, home drying, and marketing on the nutritive value of some horticultural varieties of *Beta vulgaris* and deals with the riboflavin content of Swiss chard. The high riboflavin content of leafy green vegetables makes information concerning the effect of processing on this vitamin especially important at a time when other good sources of riboflavin, such as meat, milk, and eggs, are scarce and expensive.

The literature concerning the riboflavin content of Swiss chard is limited; and that relating to the effect of cooking and dehydration on the riboflavin content of vegetables is not extensive. The riboflavin content of Swiss chard is 15 micrograms per gram of dry matter, according to Hodson (1940), who used a fluorometric method of analysis. The loss of riboflavin in processing most vegetables is evidently small. Strong, Earle, and Zeman (1941) reported that storage, cooking, or preservation of foods resulted in no considerable loss of riboflavin, and surprisingly little was removed by boiling vegetables in water. When cooked in a small quantity of water, peas, lima beans, and green snap beans lost no riboflavin—Rose and Phipard (1937) and Farrell and Fellers (1942)—while cooking increased the availability to the rat of the riboflavin of pinto beans—Lantz (1939). Oser, Melnick, and Oser (1943), on the other hand, found that fresh vegetables lost from three to 45 per cent of their riboflavin on cooking, the loss varying with the type of vegetable and being greater when the vegetable was started in a large amount of cold water than when a minimal quantity was used. Losses in riboflavin content of raw, frozen, and canned vegetables, when cooked and served in an industrial cafeteria, were 22 to 45 per cent, according to Heller, McCoy, and Lyons (1943). The work of Farrell and Fellers (1942) and that of Cruess (1943) indicates that there is little or no loss of riboflavin during the dehydration of vegetables. Farrell and Fellers (1942) found little loss even on long storage of the dried product.

In this investigation the effects of cooking, holding after cooking, home drying, and storage for marketing on the riboflavin content of two varieties of Swiss chard were studied.

EXPERIMENTAL PROCEDURE

The first paper of this series by Porter, Schlaphoff, Wharton, Briant, and Beltz (1944) gives details concerning the growing, harvesting, sam-

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pling, cooking, drying, and marketing methods used. A brief abstract of these methods follows.

Fordhook and Rhubarb chard grown on the college farm was harvested in August and September, 1942. These harvestings are referred to as Periods 1 and 2, respectively. The leaves were analyzed with midribs but without petioles. Two *home-cooking* procedures were followed, using greens equivalent to four servings. By one method, the greens were cooked uncovered in twice their weight of rapidly boiling tap water; by the other, they were cooked covered without the addition of water except that clinging to the leaves after washing. These procedures are referred to as Methods 1 and 2, respectively. The time required for cooking to a tender stage was seven to eight minutes for Method 1 and 21 to 22 minutes for Method 2. *Institution preparation* was carried out by cooking eight-pound lots of greens (approximately 30 servings) in an uncovered, steam-jacketed kettle in double their weight of rapidly boiling water for seven minutes. Cooked greens were held on the steam table for 10 and for 60 minutes. By the *home-drying* method, leaves were blanched five minutes in steam and dried with gas heat in a homemade drier for about five hours at a temperature under 70°C.(158°F.). The dried chard was cooked uncovered, without soaking, in 25 times its weight of boiling tap water. The length of time required for cooking to a tender stage was about 22 minutes. Corresponding samples of dried chard and cooked dried chard were analyzed on the same day, all the determinations being carried out within six to 15 days after drying. To show the effect of *marketing procedure*, freshly harvested leaves were rinsed, covered with wet burlap in bushel baskets, and allowed to stand at room temperature for 24 hours.

The microbiological procedure of Snell and Strong (1939) was used for estimation of riboflavin. Samples were homogenized in water in a Waring blender and autoclaved with 50 ml. of 0.1 normal HCl. The samples were ether-extracted to remove interfering fatty acids, as suggested by Strong and Carpenter (1942). Assays were made on aliquots of freshly picked leaves removed as controls from each lot to be processed. All samples of fresh and processed greens were analyzed in triplicate.

The significance of differences between the mean vitamin values was determined by analysis of variance.

RESULTS AND DISCUSSION

The riboflavin content of raw chard of two varieties, picked during two periods, is given (Table 1). Averaging data for the two periods, mean riboflavin figures of 1.69 and 2.14 micrograms per gram of fresh leaves are obtained for Fordhook and Rhubarb chard, respectively. These figures are slightly higher than the value of 1.38 micrograms per gram of fresh Swiss chard reported by Hodson (1940), using the fluorometric method. The higher value obtained in this experiment for the Rhubarb chard was not significantly different from that for Fordhook chard, according to analysis of variance. In spite of the care taken to pick greens of the same apparent maturity, the riboflavin content of chard harvested in August (Period 1) was slightly higher than that harvested in September (Period 2). These differences are not statistically significant but are

comparable to those obtained by Hunt, Record, and Bethke (1936), who found that the vitamin B_1 and G content of hays decreased as the plant matured.

The standard errors of the means for the riboflavin content of the fresh chard leaves ranged from 0.01 to 0.02, values which are not high considering the errors inherent in the microbiological method and the difficulty of sampling the chard. The chard has a large midrib which was not removed in the standard sampling procedure. To test the effect of includ-

TABLE 1

Riboflavin Content of Freshly Picked Chard [1]

Variety and period	Moisture	Riboflavin	
		Fresh leaves	Calculated to dry basis
Fordhook chard	*gm./100 gm.*	*μg./gm.*	*μg./gm.*
Period 1	91	1.77	19.6
Period 2	90	1.61	17.0
Rhubarb chard			
Period 1	89	2.42	21.6
Period 2	90	1.86	18.5

[1] Each figure represents an average of 12 determinations.

ing different proportions of midrib in the sample, assays in triplicate were conducted on one sample each of raw Fordhook and Rhubarb chard leaves and midribs. On the dry-weight basis, the leaf blade of Fordhook chard contained 19.4 micrograms, while the midrib contained only 2.72 micrograms of riboflavin per gram. Similar results were obtained for Rhubarb chard, which contained 22.6 and 3.39 micrograms per gram of dry weight in the leaf blade and midrib, respectively. This higher proportion of riboflavin in the leaf portion confirms the work of Hunt, Record, and Bethke (1936), who found that leafiness in hay was correlated with its vitamin B_1 and G content.

The effect of home cooking on the riboflavin content of chard (Table 2) indicates that the average riboflavin loss from chard cooked in a moderate quantity of boiling water was 54 per cent, while that of chard cooked with no added water was 31 per cent. The difference between these losses was significant, according to an analysis of variance calculated by using figures on the fresh-weight basis. Since riboflavin is soluble in water, it is quite possible that more of this vitamin was dissolved when a moderate quantity of water was used than when no water was added. However, no analyses of the cooking water were made, since the emphasis in this study was on the vitamin content of the vegetable as served. An additional explanation of the losses observed is suggested by the work of Williams and Cheldelin (1942) on the effects of increased pH and of temperature in aiding the photochemical destruction of riboflavin. Chard cooked in a moderate quantity of water was not covered and was immersed in rapidly boiling tap water of alkaline reaction (tap water boiled three minutes and cooled had a pH of 8.4 to 8.7). Hence, there was greater opportunity for an alkaline medium, a higher initial temperature, and more light to accelerate photochemical destruction than with chard to which no water was added.

The greater variation in the riboflavin content of cooked chard compared with fresh chard is indicated by standard errors of the mean of 0.01 to 0.03 per cent for chard cooked in a moderate amount of water and 0.01 to 0.04 per cent for that cooked without added water. This would be expected since slight variations in the cooking method as well as sampling errors undoubtedly occurred.

TABLE 2

Effect of Home and Institution Cooking on Riboflavin Content of Chard[1]

Treatment and constituents of samples	Fordhook chard		Rhubarb chard	
	Period 1	Period 2	Period 1	Period 2
Home cooking				
Riboflavin in fresh raw sample (dry wt., μg./gm., control for Methods 1 and 2)	21.5	18.4	24.2	21.9
Method 1[2]:				
Moisture (gm./100 gm.)	92	91	90	91
Riboflavin—				
As served (μg./gm.)	0.70	1.21	0.95	0.63
Dry (μg./gm.)	8.2	13.5	9.9	7.0
Loss (pct.)	62	27	59	68
Method 2[3]:				
Moisture (gm./100 gm.)	90	89	88	89
Riboflavin—				
As served (μg./gm.)	1.64	1.48	1.87	1.61
Dry (μg./gm.)	15.6	13.2	15.5	14.4
Loss (pct.)	27	28	36	34
Institution cooking				
Riboflavin in fresh raw sample (dry wt. μg./gm., control for Methods 3 and 4)	17.6	15.5	19.0	15.1
Method 3[4]:				
Moisture (gm./100 gm.)	90	92	90	91
Riboflavin—				
As served (μg./gm.)	0.64	0.57	1.09	0.90
Dry (μg./gm.)	6.6	6.6	10.8	10.1
Loss (pct.)	62	57	43	33
Method 4[5]:				
Moisture (gm./100 gm.)	89	90	88	91
Riboflavin—				
As served (μg./gm.)	0.71	0.94	1.59	0.81
Dry (μg./gm.)	6.3	9.2	13.2	8.6
Loss (pct.)	64	41	31	43

[1] Each figure represents an average of six determinations. [2] Cooked in a moderate quantity of rapidly boiling water. [3] Cooked in water clinging to the leaves after the last rinsing. [4] Held on steam table 10 minutes. [5] Held on steam table 60 minutes.

The loss of riboflavin from chard cooked by an institution method and held 10 minutes was 33 to 62 per cent, while chard held for 60 minutes lost 31 to 64 per cent (Table 2). The losses occurring during these two holding periods were not significantly different, according to an analysis of variance. Perhaps different results would have been obtained if the greens had been exposed to more light while on the steam table. The tests were carried out when dormitory meal service was not being conducted, and the light was not as bright as during cafeteria service.

The riboflavin contents of home-dried chard and of dried, reconstituted, cooked chard (Table 3) show that the average riboflavin content of all the

samples of home-dried chard was 15.6 micrograms per gram, which is higher than the value of 11.2 micrograms per gram given in a review by Cruess (1942). The total loss of riboflavin from chard during the home-drying process was seven to 39 per cent. A further loss, which was statistically significant, occurred during reconstitution and cooking, so that the chard as served had lost 40 to 63 per cent of the riboflavin content of the fresh chard. Such losses compare favorably with those which occurred while cooking the fresh chard in a moderate amount of water (Table 2). Since analyses of the dried chard were made within six to 15 days after

TABLE 3

Riboflavin Content of Home-Dried Chard, Home-Dried and Cooked Chard, and Fresh Chard Stored for Marketing[1]

Treatment and constituents of samples	Fordhook chard		Rhubarb chard	
	Period 1	Period 2	Period 1	Period 2
Home-dried				
Riboflavin in fresh raw sample (dry basis, μg./gm.)	21.5	21.3	26.6	.....
Home-dried:				
Moisture (gm./100 gm.)	7.3	7.9	10.0	.....
Riboflavin—				
Home-dried (μg./gm.)	18.4	13.9	14.6	.....
Home-dried (dry basis, μg/gm.)	19.9	15.1	16.2	.....
Loss (pct.)	7	29	39	.....
Home-dried and cooked				
Moisture (gm./100 gm.)	86	89	87	.....
Riboflavin—				
As served (μg./gm.)	1.30	1.46	1.30	.....
As served (dry basis, μg./gm.)	10.5	12.7	9.8	.....
Loss (pct.)	51	40	63	.....
Stored for marketing				
Riboflavin in fresh raw sample (dry basis, μg./gm.)	23.8	18.4	21.4	21.9
Held 24 hours at room temperature:				
Moisture (gm./100 gm.)	91	92	90	91
Riboflavin—				
After holding (μg./gm.)	2.15	1.28	2.05	1.34
After holding (dry basis, μg./gm.)	25.0	17.6	20.9	14.6
Change from fresh value (pct.)	5	—4	—2	—33

[1] Each figure represents an average of six determinations.

drying, riboflavin losses which might occur on storage of the dried product were not measured.

The change in the riboflavin content of chard held for 24 hours at room temperature in a basket under moist burlap to simulate marketing conditions varied from a gain of five to a loss of 33 per cent (Table 3). The variability of the results may have been due to variations in the room temperature.

SUMMARY

The riboflavin content of two varieties of Swiss chard, Fordhook and Rhubarb, was determined microbiologically; and the effects of cooking, holding after cooking, home drying, and storage for marketing were investigated.

The average riboflavin content of Fordhook and Rhubarb chard was 1.69 and 2.14 micrograms per gram of fresh leaves, respectively.

Chard cooked covered with no water except that clinging to the leaves after washing retained more riboflavin than chard cooked uncovered in a moderate amount of boiling water.

Chard cooked by an institution method retained about the same quantity of riboflavin when held for 10, as compared with 60, minutes.

Home-dried chard contained two-thirds or more, while dried, reconstituted, cooked chard retained about one-half of the original riboflavin. The riboflavin content of dried, reconstituted, cooked chard compared favorably with that of fresh chard cooked in a moderate quantity of boiling water.

The change in riboflavin content of chard held in a moist condition for 24 hours, as for marketing, was variable and not significant.

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ASCORBIC ACID, THIAMIN, RIBOFLAVIN, AND CAROTENE CONTENTS OF ASPARAGUS AND SPINACH IN THE FRESH, STORED, AND FROZEN STATES, BOTH BEFORE AND AFTER COOKING [1,2]

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No comparisons have been found in the literature on the ascorbic acid, thiamin, riboflavin, or carotene contents of cooked fresh, cooked stored, and cooked frozen vegetables from the same lots.

Several studies have been reported on the loss of ascorbic acid from fresh and frozen asparagus and spinach during cooking, but the results show great variation. Less information was found on the thiamin, riboflavin, and carotene contents of these vegetables in the garden-fresh and in the frozen states. Information regarding losses of these vitamins during cooking and storage is scarce.

REVIEW OF LITERATURE

Vitamin Content of Fresh Asparagus and Spinach: The ascorbic acid content of asparagus has been reported as 12.5 [4] milligrams per 100 grams by Hahn (1931); 25 [4] by Fellers, Young, Isham, and Clague (1934); 12 [4] by McHenry and Graham (1935); 25.9 by Chu and Read (1938); 18 to 44 by Fitzgerald and Fellers (1938); 20 to 30 by Thornton (1938); 16.7 by Fujita and Ebihara (1939); 100.7 and 45 by Burrell and Ebright (1940); 60 to 120 by Wolf (1940, 1941); and 43 and 55 by Jones and Fenton (1943).

The ascorbic acid content of spinach has been reported as over 50 mg. per 100 gm. by Eddy, Kohman, and Carlsson (1925); 50 by Kifer and Munsell (1932); 36.9 to 53.3 by Ranganathan (1935); 32.1 to 118.1 by Olliver (1936); 38 to 113 by Tressler, Mack, and King (1936a,b); 123.9 by Rudra (1937); 46.9 by Dunker and Fellers (1938); 19.8 by Nagel and Harris (1943); 33.8 to 69.9 by Cutlar, Jones, Harris, and Fenton (1944); and 90.4 by Dickerson (1943).

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[4] Recalculated assuming that 0.5 mg. per day is the minimum protective dose for guinea pigs.

The thiamin content of asparagus has been given as 0.195 mg. per 100 gm. by Fellers, Esselen, and Fitzgerald (1940); 0.219 by Mackinney and Sugihara (1942); and 0.183 to 0.174 by Moyer and Tressler (1943).

The thiamin content of spinach has been presented as 0.050 mg. per 100 gm. by Williams and Spies (1938); 0.108 by Fellers, Esselen, and Fitzgerald (1940); 0.126 by Conner and Straub (1941); and 0.304 by Nagel and Harris (1943).

The riboflavin content of asparagus has been presented as 0.160 mg. per 100 gm. by Fellers, Esselen, and Fitzgerald (1940) and as 0.115 to 0.113 by Mackinney and Sugihara (1942).

The riboflavin content of spinach has been found to be 0.436 mg. per 100 gm. by Fellers, Esselen, and Fitzgerald (1940) and from 0.370 to 0.460 by Munsell (1942).

The carotene content of asparagus has been given as 0.700 mg. per 100 gm. by Zimmerman, Tressler, and Maynard (1941); 0.576 [5] by Booher and Marsh (1941); and 0.480 [5] by Fellers, Young, Isham, and Clague (1934).

The carotene content of spinach has been reported as 4.30 mg. per 100 gm. by DeFelice and Fellers (1938) and as 2.460 [5] by Booher and Marsh (1941).

Effect of Storage: Jones and Fenton (1943) reported that 97 per cent of the ascorbic acid content of fresh asparagus was retained after 24 hours' storage in the hydrator tray of a mechanical refrigerator held at 1.7°C.(35°F.). Olliver (1936) found that asparagus tips lost 80 per cent of their ascorbic acid during four days' storage at room temperature. Fitzgerald and Fellers (1938) noted a 20-per cent loss of ascorbic acid from asparagus stored at 21.1°C.(70°F.) for 24 hours.

Ranganathan (1935) reported losses of 34.2 to 47.8 per cent ascorbic acid caused by storage of spinach at room temperature for 24 hours and a loss of 95.2 per cent after 192 hours. Tressler, Mack, and King (1936b) found that when spinach was stored at room temperature, from 22 to 26°C. (71.6 to 78.8°F.), for three days it lost 50 per cent ascorbic acid; when it was refrigerated at 1 to 3°C.(33.8 to 37.4°F.) for three days it retained nearly all of its original ascorbic acid. Olliver (1936) reported a loss of 78 per cent from spinach held at room temperature for two days, while storage at 0°C.(32°F.) caused slightly less destruction. Rudra (1937) noted a 19-per cent loss of ascorbic acid from spinach stored at 5°C.(41°F.) for 24 hours.

Feener, Palmer, and Fitzgerald (1937) found little change in the ascorbic acid content of fresh market spinach when stored at 1 to 3°C. (33.8 to 37.4°F.); after 72 hours at 21 to 22°C.(69.8 to 71.6°F.) it had lost from 50 to 90 per cent of its original ascorbic acid. They also reported that broken spinach leaves often contained 60 per cent less ascorbic acid than did whole leaves. Fitzgerald and Fellers (1938) found that spinach lost 43 per cent ascorbic acid when stored at 21.1°C.(70°F.) for 24 hours, and 47 per cent when thus stored for 48 hours. Cutlar, Jones, Harris, and Fenton (1944) reported a loss of 10 per cent ascorbic acid from spinach

[5] Recalculated assuming that one I. U. equals 0.6 mg. of carotene.

during the preliminary trimming, washing, and draining which extended over a five-hour period; this was preparatory to quantity cooking.

Nothing was found in the literature regarding losses of thiamin, riboflavin, or carotene from asparagus and spinach during storage.

Effect of Freezing Procedures: Fitzgerald and Fellers (1938) gave the ascorbic acid content of quick-frozen asparagus as from 9 to 32 mg. per 100 gm.; of fresh cut asparagus, not of the same lots, as from 18 to 44 mg. per 100 gm. Jones and Fenton (1943) found a 10-per cent loss of ascorbic acid on the moist basis from home-frozen asparagus which had been blanched by immersion in boiling water for three minutes. When the asparagus was blanched by steaming for four minutes, it retained almost all of its ascorbic acid. Fellers, Young, Isham, and Clague (1934) reported no loss of ascorbic acid from asparagus that had been commercially frozen.

Dunker and Fellers (1938) reported a loss of 45 per cent from spinach owing to freezing procedures. King and Tressler (1940) stated that spinach blanched in boiling water for 45 to 60 seconds lost 16 per cent, and for 153 seconds, 35 per cent ascorbic acid. Dickerson (1943) reported an average ascorbic acid loss of 49 per cent from spinach because of home-freezing procedures.

Fellers, Esselen, and Fitzgerald (1940) found that asparagus lost 22 per cent of thiamin during freezing. Moyer and Tressler (1943) reported a thiamin loss of 16 to 20 per cent from asparagus owing to freezing procedures and found the greatest loss during blanching. They found that frozen Mary Washington asparagus contained 0.204 mg. of thiamin per 100 gm.

Fellers, Esselen, and Fitzgerald (1940) reported the thiamin content of frozen spinach to be 0.102 mg. per 100 gm. There was a six-per cent loss owing to freezing procedures. Moyer and Tressler (1943) found the thiamin content of frozen King of Denmark spinach to be from 0.050 to 0.059 mg. per 100 gm.

Fellers, Esselen, and Fitzgerald (1940) reported a five per cent loss of riboflavin from asparagus and a nine-per cent loss from spinach owing to commercial freezing.

Losses of carotene from vegetables owing to freezing procedures were presented as insignificant by Fitzgerald and Fellers (1938), who determined the carotene content of 15 frozen vegetables and compared the results with values found in the literature for the fresh vegetables. Zimmerman, Tressler, and Maynard (1941) found that asparagus and spinach lost little carotene during freezing procedures. DeFelice and Fellers (1938) observed that on the moist basis spinach lost 25 per cent of its carotene during freezing procedures.

Effect of Cooking Procedures: The retention of ascorbic acid in cooked asparagus was reported as 80 per cent by Hahn (1931); 70 per cent by Fellers, Young, Isham, and Clague (1934); 68 per cent by McHenry and Graham (1935); 73 per cent by Burrell and Ebright (1940); and from 73 to 86 per cent by Jones and Fenton (1943). Olliver (1936) found the tips of cooked asparagus contained 40 per cent and the stems 70 per cent of their original ascorbic acid. Jones and Fenton (1943) reported that

reducing the amount of water below that required to cover the vegetables resulted in greater ascorbic acid retention.

The retention of ascorbic acid in cooked spinach was reported as 50 to 80 per cent by Olliver (1936); 33 to 89.7 per cent by Dunker and Fellers (1938); and 23 per cent during quantity cookery by Nagel and Harris (1943). McIntosh, Tressler, and Fenton (1940) found that frozen spinach when cooked retained from 66 to 80 per cent ascorbic acid depending upon the cooking method used.

No information was found in the literature regarding losses of thiamin or riboflavin during the cooking of asparagus.

Nagel and Harris (1943) reported that spinach cooked in quantity retained 65 per cent thiamin on the dry basis. Roscoe (1930) reported that cooked spinach lost about half of the thiamin and riboflavin in the cooking water. Hoff (1933) noted that cooked spinach contained about half as much thiamin as did raw spinach because of solution of thiamin in the cooking water. Aughey and Daniel (1940) stated that spinach lost 30 per cent thiamin during cooking; 22 per cent was destroyed and eight per cent was dissolved in the cooking water.

Crist and Dye (1929) found that raw and cooked green asparagus gave the same growth rate in rats, indicating the same carotene content in both products. Cooking of spinach was not harmful to carotene retention according to Hoff (1933), Hanning (1933, 1936), and Charley (1941).

The purpose of this study was to determine the effect on ascorbic acid, thiamin, riboflavin, and carotene contents of garden-fresh asparagus and spinach of (1) storage for 24 hours at room temperature, from 18.8 to 25.5°C.(66 to 78°F.), (2) storage for one week at 0 to 4.4°C.(32 to 40°F.), and (3) commercial quick-freezing. A second purpose was to determine whether boiling each of the above lots of vegetables in relatively large quantities of water in open pans or boiling them in small quantities of water in covered pans would result in better retention of vitamins and at the same time give a palatable product. The former method has been the usual one for cooking green vegetables.

EXPERIMENTAL PROCEDURE

History of Vegetables: The vegetables used throughout the entire study were from the same lots. They were grown in silt loam soil of the Genesee valley region in New York state. The asparagus was of Mary and Martha Washington variety and the spinach of a thick-leafed variety, Heavy Pack. The spinach was all grown within a radius of five to 10 miles from the quick-freezing plant. The asparagus was grown from one-half to one mile away. Both vegetables were part of the regular supply to be prepared and frozen for the Frosted Foods Sales Corporation.

Method of Storage and Freezing: Samples were taken from one field and one cutting for each complete study of the fresh, stored, and frozen vegetables, including the cooking of these vegetables by two methods. The vegetables were brought from the gardens to the freezing plant by truck within three to five hours after they were cut. Some of the crated, unwashed vegetables were immediately put into the cold-storage room at 0 to 4.4°C.(32 to 40°F.) to be sampled and cooked at the end of a week.

Another portion was left in the crate in the laboratory for 24 hours, at which time it was sampled and cooked. The laboratory temperature varied from 18.8 to 25.5°C.(66 to 78°F.). The vegetables were loosely packed in uncovered wooden crates and were exposed to the light naturally present in the room.

The rest of the asparagus was sent through the freezing line. A portion was taken of the medium-sized, 4¾-inch spears after washing and grading, that is, just before the blanching. This fresh asparagus was immediately sampled and cooked. It was found easier to wash and grade the fresh spinach in the laboratory instead of inferrupting the factory line to get the fresh, washed samples; however, the frozen spinach used in the study went through the regular commercial processes of cleaning, blanching, packing, and freezing.

Before being frozen the asparagus was steam-blanched for three minutes and cooled in a cold-water spray for three to four minutes; the spinach was steam-blanched for one minute and 45 seconds, and cooled in a countercurrent water bath. Both vegetables were frozen in a Birds Eye multiplate freezer.

Samples of commercially frozen asparagus and spinach for this study were stored at —40°C.(—40°F.) until they could be analyzed. It was shown by Jenkins, Tressler, and Fitzgerald (1938b) that there is little vitamin C loss in the blanched, frozen product when it is held at this temperature. Zimmerman, Tressler, and Maynard (1941) pointed out that no appreciable losses of carotene occured during storage of the blanched, frozen vegetables at either 0 or 14°F. for at least a few months. Moyer and Tressler (1943) stored samples of commercially frozen asparagus and peas for seven and five months at —12, —22, and —40°C.(10, —8, and —40°F.) and found no significant loss of thiamin from these vegetables at any of the above temperatures. No information was found regarding losses of riboflavin from frozen vegetables because of storage at low temperatures. Riboflavin is not readily destroyed except in the presence of light and heat. Therefore it would not seem likely that riboflavin would be lost from the frozen asparagus and spinach stored at —40°C.(—40°F.).

Method of Sampling: At the end of the storage period the unfrozen asparagus and spinach were washed and graded in the laboratory. Care was taken to obtain approximately the same proportion of stalk or stem as had been used in the frozen vegetable. Neither the asparagus nor the spinach was cut before cooking.

Since the frozen vegetables were taken directly from an extremely low-temperature storage room, —40°C.(—40°F.), they were held at room temperature for two hours to make temperature conditions more nearly representative of the frozen vegetables obtained from the market or locker plant. The spears of frozen asparagus were separated before cooking and the frozen spinach was cut into approximately one-inch cubes.

Barnes, Tressler, and Fenton (1943a) reported that a slight defrosting of frozen broccoli accomplished by leaving a covered package of solidly frozen broccoli at room temperature, 25.5°C.(78°F.), for one and a quarter hours resulted in no appreciable destruction of vitamin C.

Jenkins, Tressler, and Fitzgerald (1938a) and Fenton and Tressler (1938) found no appreciable loss of vitamin C during partial thawing of peas. Jones and Fenton (1943) reported that slight defrosting of home quick-frozen asparagus and broccoli, by allowing the packages to stand at room temperature of 21°C.(70°F.) for one and a half to two hours, resulted in no loss of vitamin C. Since vitamin C is the least stable of the vitamins analyzed, it is likely that no loss of thiamin, riboflavin, or carotene was caused by the slight defrosting of the vegetables before cooking.

Before each cooking, samples of the raw vegetables were taken for the determination of ascorbic acid, carotene, riboflavin, and thiamin. After cooking, both the vegetable and the cooking liquid (when there was sufficient liquid) were analyzed for the four vitamins previously mentioned. Since no appreciable amount of carotene was found in the cooking waters of either the asparagus or spinach when cooked in either the large or small amounts of water, routine samples were not taken.

After washing and grading, about 300 grams of the raw vegetable were rapidly chopped with a sharp knife on a board and well mixed. Before chopping, the asparagus spears were split lengthwise to insure a more even distribution of tips and butts. Jones and Fenton (1943) reported that tips of raw asparagus contained about twice as much ascorbic acid as did the stalks. The tips of blanched and home-frozen asparagus contained about one-fifth more ascorbic acid than did the stalks. Samples of the chopped mixture were taken immediately for vitamin analyses. The drained, cooked vegetable was chopped and sampled in the same way. In each case, duplicate samples were taken for analysis except in the determination of total solids when triplicate samples were taken.

Chemical Determinations: Ascorbic acid determinations were made according to the Bessey and King (1933) chemical-titration method as modified by Mack and Tressler (1937). Duplicate samples of both the raw and cooked vegetables were extracted with a freshly prepared solution of 1 N. sulfuric acid and two per cent metaphosphoric acid.

Suitable aliquots of the cooking water were added to chilled extractive in 50-ml. volumetric flasks and the flasks were made up to volume. Aliquots of this were titrated for vitamin C.

Aliquots of the spinach extracts from spinach that had been stored for a week were saturated with hydrogen sulfide in order to reduce any dehydroascorbic acid to ascorbic acid. The method was essentially that of Tillmans, Hirsch, and Jackisch (1932).

Thiamin determinations were made by an adaptation of the "Standard Thiochrome Assay for the Determination of Thiamin in Cereal Products" as recommended by the Research Corporation Committee on the Thiochrome Method. The method was adapted for fresh vegetables by Moyer and Tressler (1942). Twenty-five-gram samples were ground in a Waring blender with approximately 125 ml. of N/10 sulfuric acid. The size aliquot of cooking water varied with the method of cooking and the amount of cooking water available. Fifty-ml. aliquots were taken of the cooking water from the first cooking method and 10-ml. aliquots from the second cooking method. In a few cases of the latter method there was only sufficient cooking water for a five-ml. aliquot. After heating in the steam bath for 30

minutes the extract was digested with the enzyme, Clarase, for two hours at 50°C. Then the extract was made to volume, filtered, and a 50-ml. aliquot allowed to pass through the prepared Decalso column. The thiamin was removed from the Decalso by treatment with 25 ml. of 25-per cent potassium chloride in N/10 hydrochloric acid. Five-ml. aliquots of this were treated with potassium ferricyanide to produce thiochrome and then read on the photoelectric fluorometer.

Riboflavin determinations were made by the microbiological method of Snell and Strong (1939). Fifteen-gm. samples were taken in N/10 sulfuric acid, ground in a Waring blender, covered with a dark cloth and transferred to amber sample bottles. These were autoclaved at 15 pounds pressure for 15 minutes, cooled, then neutralized using methylene blue as an indicator. Asparagus extract was made up to a two-liter volume with distilled water. Spinach extract was made up to a 500-ml. volume. Fifty-ml. aliquots of the cooking water were taken from the first method of cooking and 10-ml. aliquots from the second. In a few cases by the latter method there was not sufficient cooking water for riboflavin analyses and in some instances only five-ml. samples were possible. Asparagus cooking water samples were made up to 200-ml. volume and spinach cooking water samples were made up to 500-ml. volume. One-, three-, and five-ml. aliquots of these dilutions were added to five ml. of the basal media. After autoclaving, these tubes were inoculated with *Lactobacillus casei* and allowed to incubate at 37°C.(98.6°F.) for three days and then titrated to determine the amount of lactic acid produced.

Carotene determinations were made according to the method of Zimmerman, Tressler, and Maynard (1941). Twenty-five-gram samples of asparagus and 10 grams of spinach were used. The vegetables were ground in a Waring blender with 200 ml. of hot diacetone and filtered on a Büchner funnel. After washing the residue with 100 ml. petroleum ether, the filtrates were cooled, combined, and transferred to a separatory funnel. After the various extractions and washings with petroleum ether, diacetone solution, 25 per cent potassium hydroxide in methyl alcohol, and water, the petroleum ether was dried by adding anhydrous sodium sulfate. Asparagus extracts were made up to 100-ml. volume and spinach extracts to 250-ml. volume before they were read in the colorimeter.

Total Solids: Since there was no vacuum oven available at the Snider Packing Corporation at Mount Morris, the samples taken there were finely chopped, then weighed into aluminum dry-weight dishes and placed in an oven at 98°C.(208.4°F.) for at least six hours. Then they were placed in a desiccator and taken to the Experiment Station where the drying was completed (18 hours) in a vacuum oven at 70°C.(158°F.) and 28 to 29 inches of vacuum. The samples taken in Geneva were placed directly in the vacuum oven and dried for 24 hours.

Since it was not possible to analyze the samples taken for carotene, thiamin, and riboflavin determinations immediately, these samples after being ground in the Waring blender with the extracting fluid were taken to the Experiment Station and stored at —17.8°C.(0°F.) until the analyses could be made. The maximum holding period for the carotene samples was two weeks and for thiamin and riboflavin samples, four weeks. The ascor-

bic acid determinations were made at the plant and the determinations of the total solids were started as soon as the samples were taken.

Cooking Methods: In the first cooking method for asparagus 20 times as much water was used as in the second method; in the first method for spinach 10 times as much water was used as in the second method. In both cases the larger amount of water was the maximum given in recipe books for cooking these vegetables. With the smaller amounts, danger of the vegetables boiling dry was slight and there was little excess water at the end of the cooking period (from 25 to 37 gm. for asparagus and from 31 to 109 gm. for spinach). When the larger amounts of water were used, the pan was left uncovered; with the smaller amounts, it was necessary to cover the pan to insure uniform cooking of the vegetables and to prevent excess evaporation.

In both methods, 300 gm. of vegetable were used (enough for two servings of spinach and four servings of asparagus). In the first method, each vegetable was put into a three-quart enamel stew pan, 6⅝ inches in diameter and 4 inches deep, containing rapidly boiling salted water (1,200 gm. for asparagus and 600 gm. for spinach). The water was brought back to the boil within two minutes and the vegetable was simmered until tender.

In the second method, each vegetable was placed in a 1¾-quart enamel stew pan, 6½ inches in diameter and 3 inches deep, containing 60 grams of rapidly boiling water; the pan was covered. The water was brought back to the boil within two minutes and the vegetable was simmered until tender.

The rate of evaporation during the cooking was controlled by means of a gas plate, manometer, and balance arrangement similar to that described by Fenton, Tressler, Camp, and King (1937).

The total cooking time for fresh and stored asparagus was 10 minutes and for the frozen asparagus, seven minutes. The total cooking time for fresh and stored spinach was eight minutes and for the frozen vegetable, six minutes.

Palatability Tests: In order to obtain an idea of the acceptability of vegetables cooked by these two methods, palatability tests were run for fresh and frozen asparagus and spinach. The vegetables were cooked in uncovered pans, the asparagus in 1,200 gm. of water and the spinach in 600 gm. Both vegetables were also cooked in covered pans in 60 gm. of water. These samples were labelled A and B and the judges were asked to record on a simple score card the surface appearance, color, and flavor of the two samples and to note their preference. Ten to 17 judges scored each lot of cooked vegetables.

DISCUSSION OF RESULTS

Vitamin Content of Fresh Vegetables: The garden-fresh asparagus of the Mary and Martha Washington variety contained on the fresh basis an average of 43.2 mg. of ascorbic acid, 0.187 mg. of thiamin, 0.318 mg. of riboflavin, and 0.432 mg. of carotene per 100 gm. (Table 1). On the dry basis the vitamin content was ascorbic acid, 573 mg.; thiamin, 2.583 mg.; riboflavin, 5.265 mg.; and carotene, 6.094 mg. per 100 gm.

The ascorbic acid content ranged from 42.7 to 44.1 mg. per 100 gm. on the fresh basis and from 495 to 676 mg. on the dry basis. The thiamin content ranged from 0.164 to 0.195 mg. on the fresh basis and from 2.037 to 2.916 mg. on the dry basis. The carotene content ranged from 0.430 to 0.434 mg. on the fresh basis and from 5.392 to 6.783 mg. on the dry basis.

The garden-fresh spinach of the Heavy Pack variety contained on the fresh basis 29.8 mg. ascorbic acid, 0.0867 mg. thiamin, 0.213 mg. riboflavin, and 4.063 mg. carotene per 100 gm. (Table 2). On the dry basis the vitamin content was ascorbic acid, 473 mg.; thiamin, 1.374 mg.; riboflavin, 3.372 mg.; and carotene 64.656 mg. per 100 gm.

The ascorbic acid content ranged from 20.2 to 37.9 mg. on the fresh basis. The thiamin content ranged from 0.0717 to 0.1074 mg. on the fresh basis and from 1.234 to 1.610 mg. on the dry basis. The riboflavin content ranged from 0.174 to 0.245 mg. on the fresh basis and from 2.995 to 3.673 mg. on the dry basis. The carotene content ranged from 3.795 to 4.64 mg. on the fresh basis and from 62.113 to 69.543 mg. on the dry basis.

The highest value for each vitamin was found in the asparagus and spinach analyzed at the end of the season. Similarly, Jones and Fenton (1943) and Wheeler, Tressler, and King (1939) found that broccoli harvested in the fall was higher in ascorbic acid than that harvested in the summer.

Effect of Storage: After storage in an open wooden crate at room temperature, 18.8 to 25.5°C.(66 to 78°F.), for 24 hours the asparagus lost 40 per cent ascorbic acid, no appreciable thiamin (three per cent), 22 per cent riboflavin, and nine per cent carotene (Table 1); the spinach lost 29 per cent ascorbic acid, no appreciable thiamin, no appreciable riboflavin, and little carotene (Table 2).

After storage for 24 hours at room temperature the losses of thiamin from asparagus and spinach were negligible, but the asparagus showed a marked loss, 22 per cent, of riboflavin and the spinach, five per cent. A partial explanation might be that the loss was due to the amount of light reaching the vegetables. The asparagus crate was kept on top of the laboratory desk and the vegetable was loosely packed so that direct light could reach each stalk. The spinach was stored in a crate on the floor where there was less light and the top layer of spinach kept the lower portions protected.

The losses of carotene from asparagus (nine per cent) and from spinach (eight per cent) may be considered within experimental error. Fellers (1940) stated that there was no evidence to show that short storage periods are harmful to vitamin A in foods, but longer storage periods in contact with air result in a slow but progressive loss.

After a week's storage at 0 to 4.4°C.(32 to 40°F.) the asparagus lost 57 per cent ascorbic acid, 18 per cent thiamin, 27 per cent riboflavin, and 14 per cent carotene (Table 1); the spinach lost 35 per cent ascorbic acid, 15 per cent thiamin, 17 per cent riboflavin, and no appreciable carotene (five per cent) (Table 2).

Tressler, Mack, and King (1936b) reported that upon storage for one week at 1 to 3°C.(33.8 to 37.4°F.) spinach of the Prickly Winter variety

TABLE 1

Effect of Storage and Quick-Freezing on Ascorbic Acid, Thiamin, Riboflavin, and Carotene Contents of Asparagus [1]

Condition of vegetable	Ascorbic acid			Thiamin			Riboflavin			Carotene		
	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]
	mg./ 100 gm.	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*
Garden-fresh	43.2	573	....	.187	2.583	....	.319	5.265	....	.432	6.094	....
Stored for 24 hours at 18.8 to 25.5°C. (66 to 78°F.)	27.8	342	40	.189	2.508	3	.306	4.090	22	.418	5.520	9
Stored for 1 week at 0 to 4.4°C. (32 to 40°F.)	23.7	249	57	.183	2.111	18	.324	3.847	27	.407	5.218	14
Frozen	38.7	436	24	.165	1.85	28	.269	3.030	42	.407	4.604	24

[1] The fresh, stored, and frozen asparagus were from the same lot. [2] Calculated on dry basis so as to avoid the factors of any evaporation which might have occurred during storage and any gain or loss of moisture during freezing procedures.

TABLE 2

Effect of Storage and Quick-Freezing on Ascorbic Acid, Thiamin, Riboflavin, and Carotene Contents of Spinach [1]

Condition of vegetable	Ascorbic acid			Thiamin			Riboflavin			Carotene		
	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]	Moist	Dry	Loss[2]
	mg./ 100 gm.	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*
Garden-fresh	29.8	473	....	.0867	1.374	....	.213	3.372	....	4.063	64.656	....
Stored for 24 hours at 18.8 to 25.5°C. (66 to 78°F.)	21.3	336	29	.0841	1.351	2	.197	3.205	5	3.713	59.654	8
Stored for 1 week at 0 to 4.4°C. (32 to 40°F.)	19.0	310	35	.0739	1.169	15	.171	2.808	17	3.876	61.351	5
Frozen	13.5	177	63	.0516	0.677	51	.155	2.035	40	4.269	56.053	13

[1] The fresh, stored, and frozen spinach were from the same lot. [2] Calculated on dry basis so as to avoid the factors of any evaporation which might have occurred during storage and any gain or loss of moisture during freezing procedures.

lost only about eight per cent while spinach of the Hollandia variety lost about 20 per cent of its ascorbic acid on the moist basis.

Effect of Commercial Freezing Procedures: Losses from asparagus owing to commercial freezing procedures were 24 per cent ascorbic acid, 28 per cent thiamin, 42 per cent riboflavin, and 24 per cent carotene; losses from spinach were 63 per cent ascorbic acid, 51 per cent thiamin, 40 per cent riboflavin, and 13 per cent carotene.

Both the asparagus and the spinach showed marked losses of ascorbic acid during freezing procedures. Fenton, Tressler, and King (1936) found in their studies that freezing alone did not affect the vitamin C content of peas. Jenkins, Tressler, and Fitzgerald (1938a) found that the most rapid loss of ascorbic acid from peas occurred during the cooling and washing operations subsequent to boiling-water blanching, and one-third of the loss occurred during blanching.

In general, the spinach lost a greater percentage of the water-soluble vitamins than did the asparagus. This may be at least partially explained by the greater surface area of the spinach and the rather dense surface structure of the asparagus.

The commercially frozen asparagus contained an average of 38.7 mg. ascorbic acid per 100 gm. The range was from 35.6 to 40.8 mg. per 100 gm.

The commercially frozen spinach contained an average of 13.5 mg. ascorbic acid per 100 gm. The range was from 12.0 to 14.8 mg. per 100 gm.

Home-frozen spinach of the Longstanding Bloomsdale variety which was harvested in the fall, as reported by Dickerson (1943), had a much higher ascorbic acid content (from 37.4 to 46.4 mg. per 100 gm.) than the commercially frozen spring spinach used in this study. It lost 14 per cent less ascorbic acid during freezing procedures. This difference is probably because of both varietal and seasonal differences as well as the shorter period of standing and quicker handling possible with the smaller amounts of spinach that were home frozen. Tressler, Mack, and King (1936b) found that the amount of ascorbic acid in fresh spinach varied from 38 to 62 mg. per 100 gm., depending on the variety. They also found that spinach grown in muck soil contained 50 per cent less ascorbic acid than did the same variety grown on upland soil, and that the autumn spinach contained nearly a third more ascorbic acid than did spring spinach. Similarly, Feener, Palmer, and Fitzgerald (1937) and Fitzgerald and Fellers (1938) noted that the ascorbic acid content of spinach varied with the season of growth.

The low ascorbic acid content might also be attributed to the proportion of stem to leaf used. Tressler, Mack, and King (1936b) found that the stems of spring spinach were nearly devoid of ascorbic acid but that the leaves contained from 38 to 80 mg. per 100 grams.

Effect of Two Methods of Cooking: Cooking the asparagus (Table 3) and the spinach (Table 4) in the larger amount of water in an uncovered pan resulted in a lower percentage retention of the original ascorbic acid, thiamin, and riboflavin in the vegetable than was found after cooking in the smaller amount of water in a covered pan. This occurred whether the vegetable was fresh, stored, or frozen. Similarly, Jones and Fenton (1943) found in cooking asparagus that reducing the amount of water below that

TABLE 3

Effect of Cooking in Large and Small Amounts of Water on Retention of Ascorbic Acid, Thiamin, Riboflavin, and Carotene in Fresh, Stored, and Frozen Asparagus [1]

Condition of vegetable	Method of cooking[2]			Ascorbic acid		Thiamin		Riboflavin		Carotene	
	Weight of water	Time to return to boil	Time of boiling	Retention	Solution	Retention	Solution	Retention	Solution	Retention	Solution[3]
	gm.	*min.*	*min.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Garden-fresh	1,200	2	8	81	22	71	20	78	....[4]	97	.....
	60	2	8	88	14	78	9	91	....[4]	103	.....
Stored for 24 hours at 18.8 to 25.5°C. (66 to 78°F.)	1,200	2	8	74	2	83	12	83	....[4]	91	.....
	60	2	8	77	8	89	....[4]	89	....[4]	100	.....
Stored for 1 week at 0 to 4.4°C. (32 to 40°F.)	1,200	2	8	73	15	79	17	89	4	87	.....
	60	2	8	75	7	92	....[4]	89	....[4]	101	.....
Commercially frozen	1,200	3	4	63	29	74	33	83	19	62	.....
	60	3	4	84	17	86	18	85	11	82	.....

[1] The asparagus used in this study was all from the same lot as that used in the storage and freezing studies. [2] Three hundred grams of vegetable used for each cooking. [3] Cooking water not analyzed for carotene. [4] Cooking water not analyzed.

required to cover the vegetable resulted in greater ascorbic acid retention; Barnes, Tressler, and Fenton (1943b) reported that increasing the amount of water used in cooking peas and corn increased the amount of thiamin dissolved in the cooking water and decreased the retention of thiamin in the vegetable.

The concentration of ascorbic acid, thiamin, and riboflavin was lower in the larger amount of cooking water. The cooking water from the vegetables cooked in the larger amount of water contained an average of 1.9 mg. ascorbic acid, 0.014 mg. thiamin, and 0.010 mg. riboflavin per 100 gm. from the asparagus; 4.7 mg. ascorbic acid, 0.022 mg. thiamin, and 0.044 mg. riboflavin from the spinach. The cooking water from the vegetables cooked in the smaller amount of water contained an average of 28.3 mg. ascorbic acid, 0.14 mg. thiamin, and 0.14 mg. riboflavin per 100 gm. from the asparagus; 15.8 mg. ascorbic acid, 0.068 mg. thiamin, and 0.135 mg. riboflavin from the spinach. It is likely that the housewife could more easily find use for the smaller amounts of cooking water with their higher vitamin concentration than for the larger amounts with their lower concentration.

There was probably no actual destruction of thiamin because of boiling of either vegetable (Tables 3 and 4). Barnes, Tressler, and Fenton (1943b) reported there was no destruction of thiamin during the cooking of fresh frozen peas and corn. Increasing the amounts of water used in cooking the peas and corn caused an increase in the amount of thiamin dissolved in the cooking water (16 to 36 per cent for the peas and 15 to 37 per cent for the corn). The smaller volumes of water used for cooking peas and corn were found to be more concentrated sources of thiamin than were the larger volumes.

The destruction of riboflavin was in most cases greater from the vegetable cooked in the larger amount of water (Tables 3 and 4). The increased riboflavin destruction might have resulted from the effect of light since the asparagus cooked in 1,200 gm. and the spinach cooked in 600 gm. of water were left uncovered during the cooking period.

The lower retention of riboflavin in the asparagus cooked in 1,200 gm. of water might be due only to greater solution in the cooking water; the cooking water, however, was not analyzed. When the spinach was cooked in 600 gm. of water there was not only an increase in the percentage of riboflavin found in the cooking water but also an increase of eight per cent in the destruction of riboflavin in the garden-fresh spinach, 13 per cent in that stored 24 hours at 18.8 to 25.5°C.; three per cent in that stored for one week at 0 to 4.4°C.; and three per cent in the frozen spinach. All of these percentages may be within experimental error.

Since no record was kept of the light in the laboratory at the time the vegetables were cooked, no definite conclusion can be drawn regarding the effect of light on riboflavin destruction. Since the cookings in 600 gm. and 60 gm. of water were done on the same day, at approximately the same time, it is safe to compare them.

Williams and Cheldelin (1942) reported that the light destruction of riboflavin in liquid foods proceeded at a rapid rate. The opacity of many foods, however, tends to prevent excessive riboflavin losses during cooking.

TABLE 4

Effect of Cooking in Large and Small Amounts of Water on Retention of Ascorbic Acid, Thiamin, Riboflavin, and Carotene in Fresh, Stored, and Frozen Spinach [1]

Condition of vegetable	Method of cooking[2]			Ascorbic acid		Thiamin		Riboflavin		Carotene	
	Weight of water	Time to return to boil	Time of boiling	Retention	Solution	Retention	Solution	Retention	Solution	Retention	Solution[3]
	gm.	*min.*	*min.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Garden-fresh	600	2	6	24	31	50	43	52	36	97	.....
	60	2	6	62	19	73	22	75	21	100	.....
Stored for 24 hours at 18.8 to 25.5°C. (66 to 78°F.)	600	2	6	28	50	53	54	45	39	100	.....
	60	2	6	47	19	81	25	79	18	107	.....
Stored for 1 week at 0 to 4.4°C. (32 to 40°F.)	600	2	6	34	42	50	48	52	44	99	.....
	60	2	6	52	18	82	28	82	19	99	.....
Commercially frozen	600	3	3	49	40	61	39	67	32	93	.....
	60	3	3	67	10	94	5	94	8	108	.....

[1] The spinach used in this study was all from the same lot as that used in the storage and freezing studies. [2] Three hundred grams of vegetable used for each cooking. [3] Cooking water not analyzed for carotene.

They pointed out that temperature and pH hasten the light reaction rather than directly causing chemical destruction, because when pure riboflavin solutions are heated in the dark no destruction occurs.

It is also possible that some riboflavin may have been destroyed by light during chopping and weighing of the cooked vegetable samples. No room with subdued light was available.

The quantity of cooking water did not seem to affect the percentage of carotene retained in the vegetables; almost 100 per cent of the carotene was retained in the cooked vegetable with the exception of the cooked frozen asparagus (Table 3) which retained 62 per cent when cooked in 1,200 gm. of water and 82 per cent when cooked in 60 gm. of water.

Effect of Cooking Stored Vegetables: Stored asparagus and spinach (24 hours at 18.8 to 25.5°C. and for one week at 0 to 4.4°C.) had an increased percentage of destruction of ascorbic acid during both methods of cooking over the destruction during the cooking of the fresh vegetable (Tables 3 and 4). The one exception was the fresh spinach cooked in the larger amount of water. A possible explanation of this general finding was that while there was no dehydroascorbic acid found in the cooked stored vegetables there was a slight amount (2.2 mg. per 100 gm.) in the raw stored spinach (Table 5). It may be possible that the ascorbic acid in the stored vegetable was being oxidized to the dehydro form and the cooking process completed the breakdown to the form or forms not biologically active.

TABLE 5

Effect of Storage for One Week at 0 to 4.4°C.(32 to 40°F.) on Reduced and Total Ascorbic Acid Content of Raw and Cooked[1] Spinach

Condition of vegetable when sampled	Amount of cooking water	Ascorbic acid			
		Reduced		Total	
		Vegetable	Cooking water	Vegetable	Cooking water
	gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*	*mg./ 100 gm.*
Raw, fresh	.....	29.8	.....	.....	.....
Raw, stored one week	.....	19.0	.....	21.2	.....
Cooked	600	9.2	3.7	9.4	3.2
Cooked	60	15.6	12.4	16.0	12.7

[1] Three hundred grams were cooked for eight minutes.

In general the retention of thiamin and riboflavin in the cooked stored asparagus and spinach was higher than that in the cooked fresh vegetable (Tables 3 and 4).

Fyler and Manchesian (1938) measured the rate of leaching of magnesium, calcium, and phosphorus from asparagus and peas during steaming and boiling after storage at 1.7°C.(35°F.) for various lengths of time. They found that peas and asparagus harvested and stored at 35°F. for more than 48 hours retained more of their mineral constituents when cooked than did those cooked immediately after harvesting. They found that the crude fiber content of uncooked peas at the various storage periods increased measurably with storage. The crude-fiber content of the asparagus did not vary significantly.

Effect of Cooking Frozen Vegetables: Frozen spinach during cooking procedures retained, in general, a slightly higher percentage of ascorbic acid, thiamin, and riboflavin than was retained by the cooked, garden-fresh spinach. This was not true of the asparagus. When cooked in the larger amount of water the fresh asparagus retained 81 per cent ascorbic acid and the frozen asparagus, 63 per cent. When cooked in the smaller amount of water, the fresh asparagus retained 88 per cent ascorbic acid and the frozen asparagus, 84 per cent. When cooked in the larger amount of water the fresh spinach retained 24 per cent ascorbic acid and the frozen spinach, 49 per cent. When cooked in the smaller amount of water the fresh spinach retained 62 per cent ascorbic acid and the frozen spinach retained 67 per cent (Table 4).

The thiamin retention was three per cent higher and the riboflavin retention five per cent higher in the frozen asparagus cooked in the larger amount of water; and eight per cent higher and six per cent lower, respectively, in that cooked in the smaller amount of water than the percentage retention of thiamin and riboflavin in the cooked fresh asparagus. Owing to the experimental error of the methods for thiamin and riboflavin determinations the differences may be insignificant.

The thiamin retention was 11 per cent higher and the riboflavin retention 15 per cent higher for the frozen spinach cooked in the larger amount of water, and 21 per cent and 19 per cent higher, respectively, in that cooked in the smaller amount of water, than the percentage retention of thiamin and riboflavin in the cooked fresh spinach.

Losses of carotene during the cooking of frozen asparagus were 38 per cent in the larger amount of water and 18 per cent in the smaller amount of water. These losses seem unusual since there was no apparent loss of carotene during cooking from the garden-fresh or stored asparagus or from the garden-fresh, stored, or frozen spinach. This loss might be due to incomplete blanching of the asparagus so that the carotene was destroyed by enzyme activity during the initial stages of cooking, or possibly to the softening of the tissues of the asparagus by the freezing process so that some carotene was dispersed into the cooking water.

Palatability: The judges found little difference in the color and surface appearance of fresh asparagus and fresh spinach when cooked by either method. Some noted that the asparagus cooked in the smaller amount of water was slightly more brown than was the asparagus cooked in the larger amount of water.

The texture of the vegetables cooked in a larger amount of water was scored as slightly softer. Probably the texture of the two lots could have been made more similar by adjusting the cooking time.

Flavors of the vegetables cooked in more water were judged weaker than those in less water. Some judges, the majority of whom were accustomed to having their vegetables rather well done, gave a slight preference to the milder flavored, softer vegetables. The women judges, who were in the minority, preferred the vegetables cooked in the smaller amount of water.

The judges had great difficulty in deciding in favor of one method or the other and some could make no choice.

The palatability of the frozen vegetables cooked by the two methods rated about the same: six judges gave no preference, six preferred those cooked in the larger amount of water, and five preferred those cooked in the smaller amount of water. None of the judges could observe any difference in the color of either the frozen asparagus cooked by the two methods or of the frozen spinach cooked by the two methods.

SUMMARY

Garden-fresh Mary and Martha Washington asparagus contained on the fresh basis 43.2 mg. of ascorbic acid, 0.187 mg. of thiamin, 0.318 mg. of riboflavin, and 0.432 mg. of carotene per 100 grams.

Garden-fresh, spring-harvested, Heavy Pack spinach contained on the fresh basis 29.8 mg. of ascorbic acid, 0.087 mg. of thiamin, 0.213 mg. of riboflavin, and 4.063 mg. of carotene per 100 grams.

During storage for 24 hours at 18.8 to 25.5°C., asparagus lost 40, 3, 22, and 9 per cent and spinach lost 29, 2, 5, and 8 per cent of the ascorbic acid, thiamin, riboflavin, and carotene, respectively.

During storage for one week at 0 to 4.4°C., asparagus lost 57, 18, 27, and 14 per cent and spinach lost 35, 15, 17, and 5 per cent of the ascorbic acid, thiamin, riboflavin, and carotene, respectively.

During freezing procedures, asparagus lost 24, 28, 42, and 24 per cent and spinach lost 63, 51, 40, and 13 per cent of the ascorbic acid, thiamin, riboflavin, and carotene, respectively.

During cooking in a large amount of water in an uncovered pan, fresh asparagus retained 81, 71, 78, and 97 per cent and fresh spinach retained 24, 50, 52, and 97 per cent of the ascorbic acid, thiamin, riboflavin, and carotene, respectively. During cooking in a small amount of water in a covered pan fresh asparagus retained 88, 78, 91, and 100 per cent and fresh spinach retained 62, 73, 75, and 100 per cent of the ascorbic acid, thiamin, riboflavin, and carotene, respectively.

Cooking the fresh, stored, and frozen spinach in a relatively large amount of water in an open pan resulted in a greater loss of ascorbic acid, thiamin, and riboflavin than did cooking them in a small amount of water in a covered pan. The trend in the cooking of asparagus was similar but not so marked. The quantity of cooking water made no appreciable difference in carotene retention.

Cooking the fresh, stored, and frozen asparagus and spinach in a large amount of water resulted in a greater per cent solution of ascorbic acid, thiamin, and riboflavin than did cooking in the smaller amount of water, but the concentration of these vitamins in the cooking water was lower.

There was no appreciable destruction of thiamin owing to cooking of asparagus and spinach in either the large or the small amount of water.

Cooking spinach in a small amount of water in a covered pan resulted in slightly less riboflavin destruction than did cooking in a large amount of water in an open pan.

There was practically complete retention of carotene in the cooked vegetables with the exception of the frozen asparagus.

During cooking the stored vegetables lost a greater percentage of ascorbic acid but a smaller percentage of thiamin and riboflavin than did the fresh vegetables.

Both the asparagus and the spinach cooked in the larger amounts of water were more bland in flavor than were those samples cooked in the small amounts. The judges were about equally divided, however, in their choice of methods and some could not differentiate between them.

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PRELIMINARY STUDIES ON STABILIZATION OF THIAMIN IN DEHYDRATED FOODS[1]

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Dehydrated and canned products of all types frequently are subjected to unfavorable storage owing partly to unavoidable shipping and warehouse conditions and partly to the assumption that such foodstuffs are entirely stable. The fact that foods of these types can survive long periods under adverse conditions without definite spoilage contributes to the erroneous belief that they are nutritionally stable. Unfortunately neither dehydrated nor canned foods retain their nutritive value completely under all conditions. In some products there are serious losses of vitamins, especially thiamin, during storage unless certain precautions are observed. The general acceptance of pork products as excellent sources of thiamin emphasizes the importance of this problem in dealing with dehydrated meats.

Usually losses of thiamin during storage are negligible at low temperatures, but above ordinary room temperatures losses in either dehydrated or canned products often become great. It has been reported previously that the thiamin content of dehydrated pork decreases rapidly during storage at temperatures above 37°C.(98.6°F.)—Rice and Robinson (1944a). This is not true of dehydrated pork alone since dehydrated eggs, Klose, Jones, and Fevold (1943); canned meats, Rice and Robinson (1944b); canned green beans, Farrel and Fellers (1942); and condensed milk, Knott (1942) also lose thiamin at appreciable rates while stored at temperatures ranging from 25 to 49°C.(77 to 120.2°F.).

On the other hand, skim-milk powder, wheat, soya flour, dehydrated vegetables, and certain food mixtures appear to be much more stable (Table 2). Inasmuch as some foods seem to have their thiamin "stabilized" to a significant degree, it seemed possible that they might exert a "protective" action upon the thiamin in the less stable foods if mixed with the latter. Such an effect seems to be responsible for the greater stability of thiamin in certain meat-cereal mixtures—Rice, Beuk, and Robinson (1943).

Whether a chemical substance is responsible for the stabilizing action or whether the effect is due to a physical influence cannot be ascertained from existing information. The studies reported here were conducted with the hope that the factors affecting thiamin retention might be defined more clearly.

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[1] Presented before the Division of Biological Chemistry, American Chemical Society, at Pittsburgh, September, 1943.

EXPERIMENTAL PROCEDURE

General Procedures: A modification of Hennessy and Cerecedo's thiochrome procedure was used for determining thiamin—Hennessy and Cerecedo (1943). Measurements of thiochrome intensities were made with a Coleman Model 11 Photofluorometer.

Since it had been suggested that thiochrome procedures do not determine all of the thiamin in dehydrated food samples—Lane, Johnson, and Williams (1942)—it seemed advisable to check this method against biological assays. As is shown later, the two methods agreed within the limits of experimental error.

Each food item used in the stability tests was finely ground and thoroughly mixed. Control samples were stored at —29°C.(—20.2°F.) for the same period of time as the test samples stored at higher temperatures. Control and test samples were analyzed simultaneously, thus avoiding errors owing to variations in standard, instrument, or technique. Retentions in the test samples were expressed as a percentage of the thiamin in the control samples, on a solids basis.

TABLE 1

Loss of Thiamin in Dehydrated Pork at Various Temperatures

Storage temperature	Initial value	Thiamin retained		
		7 days	14 days	21 days
°C.	*μg./gm.*	*pct.*	*pct.*	*pct.*
—29	14.5	100	100	100
3	14.5	100	100	96
27	14.5	.....	89	77
37	14.5	70	55	43
49	14.5	15	7	0
63	14.5	4	0	0

After initial experiments had indicated rates of loss at different temperatures (Table 1), test samples were held at 49°C. in a constant temperature oven. This very severe treatment was adopted in order to obtain high losses in a short period of time. It is far above the usual temperatures encountered in handling foods and hence exaggerates the actual losses in commercial products.

Rate of Loss of Thiamin in Dehydrated Pork Stored at Various Temperatures: Exploratory experiments showed that thiamin is readily destroyed in dehydrated pork held at 37°C. Therefore, it seemed desirable to determine the rates at which losses would occur at other temperatures. A single, well-mixed lot of dehydrated pork was vacuum-packed in one-pound cans and stored at the temperatures and for the intervals indicated (Table 1).

From the results it is obvious that temperatures above 27°C.(80.6°F.) promote rapid loss of thiamin and that storage for any reasonable length of time under such conditions will result in the destruction of most of this vitamin.

Retention of Thiamin in Various Dehydrated Products: In order to provide comparative data, a number of other foods were packaged in

cork-stoppered test tubes and stored along with the dehydrated pork samples. Since these other foodstuffs are not customarily vacuum-packed, it was considered unnecessary to so pack them in this experiment which was intended to determine losses under conditions comparable to commercial practice.

Although the moisture content of these samples varied somewhat, moisture does not seem to be correlated directly with thiamin stability. Dehydrated pork and wheat (each containing eight to 10 per cent water) showed marked differences in thiamin retention despite similar moisture levels. The same is true of dehydrated eggs and skim-milk powder, both of which contained four to five per cent moisture. Nevertheless, in subsequent experiments all samples were adjusted to six per cent moisture in order to eliminate this variable.

TABLE 2

Retention of Thiamin in Dried Products Stored 21 Days at 49°C.(120.2°F.)

Product	Thiamin
	pct.
Dehydrated skim milk	100
Dehydrated meat-cereal mixture [1]	94
Ground whole wheat	85
Dehydrated eggs	35
Dehydrated pork	10

[1] Cracked wheat, 34.2 per cent; ground barley, 21.7 per cent; soya flour, 27.6 per cent; bone meal, 7.2 per cent; dried skim milk, 5.5 per cent; salts, 2.3 per cent; tomato paste, 1.65 per cent; sardine oil, 0.25 per cent; and gum guaiac, 0.16 per cent.

The stabilities of these products differ greatly, as may be noted from the data obtained (Table 2). The excellent retention of thiamin in a dehydrated meat-cereal mixture—Rice, Beuk, and Robinson (1943)—containing 67 per cent of meat products is in sharp contrast to the great loss in dehydrated pork and illustrates the differences in stability that may be encountered in products more or less similar in composition.

Effectiveness of Various Substances in Stabilizing Thiamin in Dehydrated Pork: Since the dehydrated meat-cereal mixture lost thiamin slowly regardless of the fact that it contained approximately 67 per cent of meat, it is apparent that the non-meat portion [2] must be responsible for the stability. To determine if this cereal mixture would have the same effect on other meats, especially pork, all of the non-meat ingredients were combined with lean, ground pork in the proportions indicated (Table 3). In one sample sodium nitrite was omitted. These preparations were then cooked and dehydrated to six per cent moisture under identical conditions.[3] Portions of the same ground pork used in preparing the pork-cereal mixture were dehydrated alone and in combination with various individual

[2] Cracked wheat, 34.2 per cent; ground barley, 21.7 per cent; soya flour, 27.6 per cent; bone meal, 7.2 per cent; dried skim milk, 5.5 per cent; salts, 2.3 per cent; tomato paste, 1.65 per cent; sardine oil, 0.25 per cent; and gum guaiac, 0.16 per cent. For convenience, this mixture will be referred to as "cereal mixture," although it contains non-cereal materials.

[3] All products were cooked for 15 minutes at 10 pounds pressure and at 116°C. (240.8°F.).

non-meat ingredients of the cereal mixture. Samples of the various preparations were held at 49°C. for seven days for comparison with controls stored at —29°C.

There was significant improvement in thiamin retention when 33 per cent of the cereal mixture was dehydrated with the pork. When smaller amounts of this mixture or of the individual constituents were used, there was much less stabilization. The omission of sodium nitrite from the cereal mixture did not reduce its effectiveness. Because of the significance of these experiments they were repeated with similar results.

TABLE 3

Effect of Different Amounts and Ingredients of Cereal Mixture on Stability of Thiamin in Dehydrated Pork[1]

Added amount or ingredient	Thiamin retained 7 days at 49°C. (120.2°F.)	
	Run 1	Run 2
	pct.	*pct.*
None	15	10
Cereal mixture, 33%	96	75
Cereal mixture, 22%	22	34
Cereal mixture, 11%	11	32
Cereal mixture without $NaNO_2$, 33%	100	....
Wheat, 11.5%	37	....
Barley, 7.5%	39	....
Soybean flour, 9.6%	33	20
Tomato paste, 1%	37	28
Tomato paste, 5%	....	16
Soybean flour, 20% + tomato paste, 2%	....	35

[1] All samples contained six per cent moisture.

From these data it is apparent that the cereal mixture exerted a stabilizing effect upon the thiamin in the mixed product. Dehydrated pork alone retains 10 to 15 per cent of its original thiamin for seven days at 49°C. The same pork plus 33 per cent of the cereal mixture retains 75 to 97 per cent, six times as much as the pork alone. Numerous other comparisons not recorded here substantiate these ratios.

That the effect noted is not associated specifically with any one of the major ingredients is indicated by their failure to stabilize thiamin in pork when dehydrated with it in approximately the same proportions as when included in the cereal mixture. Thus, meat dehydrated with 11.5 per cent wheat failed to show much stabilization. Similarly, no other individual ingredient improved retention markedly when present in the same concentration as in the cereal mixture. However, when 33 per cent of wheat, barley, soybean flour, or dried skim milk were cooked and dehydrated with pork, the stabilization was as pronounced as that resulting from the use of a similar quantity of the entire cereal mixture (Table 4). It thus appears that a number of materials are capable of stabilizing thiamin if included in sufficient quantities.

The stabilization affects the thiamin in the meat as well as that in the cereal, since retention in a meat-cereal mixture is better than the sum of the individual retentions. One hundred grams of the pork-wheat mixture

referred to (Table 4) contained 1.13 mg. of thiamin. Of this, pork contributed 1.04 mg. while wheat supplied only 0.09 mg. If the wheat and pork had been stored separately, retentions at the end of the storage period would have been 0.21 mg. (1.04 x 20 per cent) in the pork and 0.08 mg. (0.09 x 85 per cent) (Table 2) in the wheat, or a total of 0.29 mg. The cooked and dehydrated mixture, however, retained 0.99 mg. (1.13 x 85 per cent) or more than three times as much as the sum of individual retentions. Thus, the wheat obviously influences the rate of thiamin destruction in the meat.

TABLE 4

Effect of Ingredients of Cereal Mixture on Stability of Thiamin in Dehydrated Pork

Addition	Thiamin retained 7 days at 49°C.(120.2°F.)	pH
	pct.	
None	20	6.30
Cereal mixture, 33%	89	6.25
Wheat, 33%	88	6.15
Barley, 33%	88	6.08
Soybean flour, 33%	75	6.38
Soybean flour, wheat, and milk, 11% each	88	6.29
Dried skim milk, 33%	88	6.30
Bone meal, 5%	23	.....

Since samples showing a wide range of stability had similar pH values (Table 4), there is no indication that differences in acidity are responsible for the various rates of thiamin loss.

Various substances known to stabilize other constituents of foods were not very effective in stabilizing thiamin in dehydrated pork. In addition to the substances listed (Table 5), lecithin and thiourea were also ineffective.

TABLE 5

Effect of Various Substances in Stabilizing Thiamin in Dehydrated Pork

Substance added	Thiamin retained 7 days at 49°C.(120.2°F.)
	pct.
None	11
Nipa, 0.1%	20
NaCl, 5%	3
Gum guaiac, 1%	15
Avenex, 2%	27
Avenex, 5%	20
Avenex, 10%	28
Glucose, 5%	23
Dried milk, 5%	20

Influence of Atmosphere Within Package on Thiamin Stability: That atmospheric oxygen is not responsible for the rapid loss of thiamin from pork was demonstrated by determining the thiamin content of replicate samples after storage at 49°C. for one week in vacuum, air, nitrogen, or

carbon dioxide (Table 6). In fact, the retention of thiamin was slightly greater in the samples stored in air at 49°C. than under the other conditions. Even though there may be slightly improved retention of thiamin by storage in air, the development of rancidity makes such a procedure impractical.

TABLE 6

Effect of Storage Conditions on Thiamin Retention

Storage condition	Thiamin retained 7 days at 49°C.(120.2°F.)	
	Run 1	Run 2
	pct.	*pct.*
Packed in vacuum	2	0
Packed tightly in air	15	14
Packed loosely in air	28	....
Packed loosely in CO_2	11	....
Packed loosely in N_2	0	....

Influence of Moisture on Thiamin Stability: Although moisture is not the sole factor determining the stability of thiamin, as pointed out above, under certain conditions it greatly influences the rate of loss. To determine the extent of its influence, dehydrated pork was held in a vacuum over phosphorus pentoxide until its weight became constant. Samples of various moisture levels were then prepared and stored at 49°C. for a week. Anhydrous pork retained its thiamin well; but as the moisture content increased, the rate of loss also increased until at six per cent it had reached a maximum (Table 7). This makes it seem possible that moisture is one of the factors involved. On the other hand, canned pork with a moisture content of 50 to 55 per cent is much more stable than dehydrated pork, losing only 15 to 18 per cent of its thiamin in seven days at 49°C., Rice and Robinson (1944b), a striking contrast to the 85 to 90 per cent loss ordinarily shown in dehydrated pork. It is obvious, therefore, that moisture content *per se* is not the sole factor involved in thiamin breakdown, although complete dehydration is one means of lessening thiamin loss. From a commercial standpoint the production of anhydrous pork is impractical.

Biological Analyses: Male Sprague-Dawley rats initially weighing 35 to 45 grams were fed a thiamin-free diet [4] until they showed growth failure and polyneuritic symptoms (20 to 25 days). The rats were then distributed into six groups in accordance with the principles prescribed by the U. S. Pharmacopoeia XII, each group consisting of 10 individually caged rats. In addition to the basal thiamin-free diet (fed *ad libitum*) daily supplements as indicated (Table 8) were administered for 28 days. These supplements were readily consumed when mixed with small portions of

[4] The thiamin-free diet was prepared by treating 10-pound quantities of the "meat-cereal mixture" (which has been used in this laboratory with excellent success in raising rats) with 167 grams of Na_2SO_3 dissolved in a liter of water, acidifying with HCl to pH 5.5, and then autoclaving for one hour at 15 pounds pressure. The sulfite-treated material was then dried in a cabinet drier with air at 63°C.(145.4°F.). Thiochrome analyses indicated the product to be free of thiamin.

the basal diet and placed on top of the regular feed. The quantities of meat to be fed daily to provide each rat with four micrograms of thiamin were calculated from thiochrome assays which indicated the fresh pork to contain 9.6 micrograms of thiamin per gram and the dehydrated pork No. 184 and No. 184S to contain 14.5 and 1.0 micrograms per gram, respectively.

TABLE 7

Influence of Moisture Content on Stability of Thiamin

Moisture [1]	Thiamin retained 7 days at 49°C. (120.2°F.)
pct.	*pct.*
0	91
2	60
4	23
6	9
9	11

[1] All samples sealed *in vacuo*.

Pork No. 184S was a portion of No. 184 which had been held for two weeks at 49°C. Since direct biological analysis of No. 184S would have necessitated the feeding of large supplements, it was fed at the same level as No. 184 with sufficient pure thiamin added to make the thiamin intake four micrograms per day, assuming the thiochrome values to be correct. While this procedure cannot be expected to yield an exact analysis of the product, it is a sensitive way of detecting "bound" thiamin.

All groups receiving the calculated four micrograms of thiamin in fresh or dehydrated pork grew at a rate equal to the group fed four micrograms of pure thiamin (Table 8). Therefore, within experimental error, the two methods check. The failure of the group receiving the stored dehydrated pork plus pure thiamin to grow more rapidly than the control group proves that the low thiamin values found for such samples by thiochrome analyses are not more than a few per cent in error.

DISCUSSION

Although definite reduction in the loss of thiamin in dehydrated pork may be achieved by the use of high levels of cereals or dried skim milk, the mechanisms involved are rather obscure. Since thiamin is quite readily inactivated by hydrolytic cleavage, it might be assumed that water is responsible for its destruction in foods. The experiment showing the influence of reducing the water content of dehydrated pork from six per cent to 0 supports such a belief, but the great stability of thiamin in other foods containing as much as 50 per cent water shows that water content *per se* does not determine the degree of destruction of thiamin. However, certain substances, such as the carbohydrates or proteins which are present in cereals and dried milk, may have chemical or physical properties which enable them to inhibit the action of water on thiamin. The predominance of carbohydrate material in the stabilizing substances so far studied indicates that the factor may be associated in some manner with carbohydrate. This is made to seem even more probable since a recent experiment has proved cornstarch to be as effective as the cereals in stabilizing thiamin.

Work now in progress should determine whether or not such a conclusion is justified.

It seems rather improbable that the loss of thiamin from dehydrated pork stored at elevated temperatures is caused by enzymic hydrolysis of the nature postulated by Sealock, Livermore, and Evans (1943), since pork is cooked prior to dehydration; and these workers and others—Green, Carlson, and Evans (1941) and Woolley (1941)—studying the same phenomenon, i.e., the inactivation of thiamin by fresh-fish tissues, have demonstrated the heat lability of the thiamin-destroying factor in their preparations. It is possible, however, that even after cooking, traces of the original enzymic activity remain and that this is responsible for the effects here reported. In such cases, the excess of carbohydrate or some substance occurring with it might inhibit the reaction between thiamin and the enzyme.

TABLE 8

Biological Analyses of Pork Products

Group No.	Daily supplement	Average gain in 28 days	Thiamin intake, calculated from gains
		gm.	*μg.*
1	None	11.6[1]	.....
2	6 micrograms crystalline thiamin	76.6	6.00
3	4 micrograms crystalline thiamin	60.5	4.00
4	0.414 gm. fresh pork containing 4 micrograms thiamin[2]	59.5	3.86
5	0.276 gm. dehydrated pork, No. 184, containing 4 micrograms thiamin[2]	63.9	4.41
6	0.276 gm. dehydrated pork, No. 184S, containing 0.28 micrograms thiamin[2] plus 3.72 micrograms pure thiamin	61.6	4.13

[1] Five survivors. [2] Measured by thiochrome method.

SUMMARY

It has been shown that dehydrated pork and dehydrated eggs stored at or above temperatures of 37°C. (98.6°F.) lose thiamin more rapidly than certain other foods, such as dried skim milk or cereals.

Dehydrated mixtures of 67 per cent pork and 33 per cent cereals (wheat, barley, or soya flour) or dehydrated milk lose thiamin much less rapidly than dehydrated pork alone.

The rate of loss of thiamin from dehydrated pork is roughly proportional to the moisture content of the sample between 0 and six per cent moisture levels. Canned pork, with moisture of 50 to 55 per cent, shows

less loss than dehydrated pork with only two per cent moisture, so factors other than this are involved in thiamin stability.

Storage of dehydrated pork in vacuum, nitrogen, or carbon dioxide rather than in air did not increase thiamin retention.

In levels as great as or greater than ordinarily used in foods, Avenex, Nipa, lecithin, glucose, and sodium chloride were ineffective in reducing thiamin loss.

Products showing greatly different stabilities may have similar pH values.

Products rich in carbohydrate materials are the most effective stabilizing agents so far studied.

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VITAMIN A CONTENT OF PALM OILS

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Palm oil is obtained from the fruit of the palm. Since palm oil is a potential source of vitamin A, its use in food products may increase. For this reason, investigation of the vitamin A content of the oil was started in this laboratory in 1932. One of the earlier investigations by the authors, Poe and Fehlmann (1933a), demonstrated the value of palm oil in oleomargarines as a carrier of vitamin A.

In order to obtain samples from widely different sources letters were written to many of the large importers of oils and to the American consuls in several countries. Thirty-two samples of crude oil and seven samples of refined oil were obtained. The majority of the samples were from Africa, Sumatra, and the Federated Malay States; the sources of the various oils are listed (Tables 1 and 2).

The methods and procedures used in the investigation of the vitamin content of palm oil were essentially those recommended by Sherman and Munsell (1925) and Sherman and Burtis (1928) and have been used with entire satisfaction in previous investigations by Poe and Fehlmann (1933b); Poe, Gant, and Griffin (1939); and Fehlmann (1940). The detailed procedures will not be outlined in the present communication.

The vitamin A content of the various samples of crude and refined palm oil are presented (Tables 3 and 4); growth curves for the 32 samples of crude palm oil are shown (Fig. 1) and for the seven samples of refined palm oil, (Fig. 2). A study of the tables and charts shows that many samples of crude palm oil contained a large amount of vitamin A; however, there was a wide variance in the content of different samples. The units of vitamin A varied from 12.3 in sample No. 28 to 419.4 in sample No. 14. The average was 132.5 units per gram.

The amount of vitamin in the refined oil was very low in all the samples except No. 5, which contained 233.4 units. The rather large amount of vitamin in the crude oils compared with the small amount in the refined samples suggests that the process of refining the oils may be responsible for the loss of the vitamin.

The authors wish to thank the companies which furnished the samples. Also appreciation is expressed to Mr. L. H. Gourley and to Mr. Lester Maynard, both of the United States Consular Service, for their kindness in locating several sources and obtaining samples of palm oil.

TABLE 1

Sources of Crude Palm Oils

Sample No.	Source	Donor
1	Africa	United Africa Co., Inc., New York, N. Y.
2	Africa	United Africa Co., Inc., New York, N. Y.
3	Africa	United Africa Co., Inc., New York, N. Y.
4	Africa	United Africa Co., Inc., New York, N. Y.
5	Nigeria	Lever Brothers Co., Cambridge, Mass.
6	Nigeria	Lever Brothers Co., Cambridge, Mass.
7	Belawan-Deli, Sumatra	Lever Brothers Co., Cambridge, Mass.
8	West Africa	Commercial sample
9	Sumatra	Stein, Hall and Co., New York, N. Y.
10	Sumatra	Durkee Famous Foods, Inc., Chicago, Ill.
11	Unknown	Commercial sample
12	Unknown	Commercial sample
13	Unknown	Proctor and Gamble Co., Ivorydale, Ohio
14	Sumatra	Best Foods, Inc., New York, N. Y.
15	West Africa	Best Foods, Inc., New York, N. Y.
16	Poeloe Radja, Sumatra	United Serdang Rubber Plantations, Goenoeng Malajoe Factory
17	Rampah, Sumatra	Rambong Sialang estate
18	Batang, Berjuntai, Selangor, Federated Malay States	Tennamaram estate
19	Batang, Berjuntai, Selangor, F. M. S.	Sungei Tinggi estate
20	Sungei, Buloh, Selangor, F. M. S.	Elmina estate
21	Batang, Berjuntai, Selangor, F. M. S.	Hopeful estate
22	Serdang, Selangor, F. M. S.	Government Experiment Plantations
23	Klang, Selangor, F. M. S.	Highlands estate
24	West Africa	Colgate-Palmolive-Peet Co., Kansas City, Kan.
25	West Africa	Colgate-Palmolive-Peet Co., Kansas City, Kan.
26	West Africa	Colgate-Palmolive-Peet Co., Kansas City, Kan.
27	Sumatra	Unknown
28	West Africa	Commercial sample
29	Sumatra	Commercial sample
30	Rampah, Sumatra	Mata Pao estate
31	Pematang Siantar, Sumatra	Marihat Sumatra Plantagen Co.
32	Africa	United Africa Co., Inc., New York, N. Y.

TABLE 2

Sources of Refined Palm Oils

Sample No.	Source	Donor
1	Unknown	Swift and Company, Chicago, Ill.
2	Unknown	Commercial sample
3	Unknown	Durkee Famous Foods, Inc., Chicago, Ill.
4	Unknown	Best Foods, Inc., New York, N. Y.
5	Unknown	Proctor and Gamble Co., Ivorydale, Ohio
6	Rampah, Sumatra	Mata Pao estate
7	Sumatra	Best Foods, Inc., New York, N. Y.

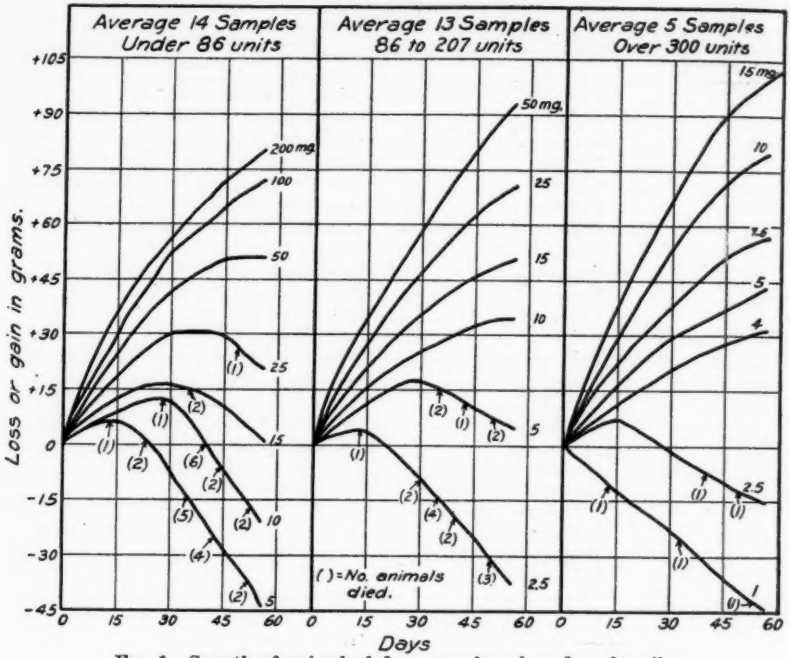

FIG. 1. Growth of animals fed on samples of crude palm oil.

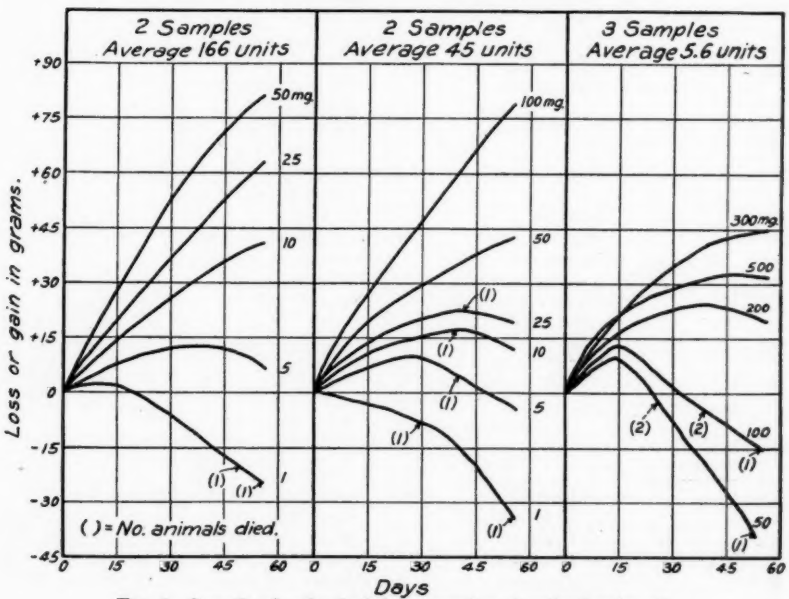

FIG. 2. Growth of animals fed on samples of refined palm oil.

TABLE 3

Units of Vitamin A in Samples of Crude Palm Oil

No.	Units per gram
1	32.1
2	206.5
3	187.5
4	156.4
5	38.3
6	55.9
7	124.7
8	316.7
9	321.0
10	133.5
11	20.9
12	43.4
13	47.9
14	419.4
15	108.4
16	357.2
17	57.8
18	44.2
19	57.2
20	70.1
21	194.5
22	144.5
23	107.6
24	122.3
25	354.4
26	27.0
27	80.9
28	12.3
29	102.2
30	85.9
31	175.0
32	34.2
Average	132.5

TABLE 4

Units of Vitamin A in Samples of Refined Palm Oil

No.	Units per gram
1	60.0
2	0.6
3	4.7
4	30.0
5	233.4
6	98.0
7	11.8
Average	62.6

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STUDIES ON THE BACTERIOLOGY OF STORED, DRIED EGG POWDER[1]

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With ever-larger quantities of dried egg powder being used by the armed forces and lend-lease, problems relating to control of the quality of the product become very important. The total numbers of bacteria in the fresh product are but one standard by which the quality of whole-egg powder is measured. As the product is stored the significance of the bacterial flora at a particular time is difficult to appraise. Some emphasis must first be placed upon the bacterial flora of fresh liquid eggs, and the powder made therefrom, before the bacteriology of stored egg powder can be interpreted.

The work of DeBord (1925), Stuart, Hall, and Dicks (1942), Funk (1943), and Gibbons and Fulton (1943) indicates that the bacteria present in the fresh, dried egg powder is largely determined by the following factors: (1) the number and kind of bacteria in the liquid whole egg, (2) the temperature to which the product is subjected while being processed and then stored, (3) the activity of lysozyme, (4) whether or not the eggs are pasteurized before being broken out, (5) the evenness as well as the method of drying, and (6) the amount of moisture in the finished product. Obviously, insanitary conditions in the plant and carelessness in breaking and handling the eggs would contribute still more organisms to the product. However, there appears to be general agreement among investigators that the lower the total bacterial count in the liquid eggs just prior to drying, the higher will be the quality of the dried egg powder. To accomplish this purpose Mallmann and Churchill (1942) have recommended a procedure for following the eggs through the plant with the help of a direct microscopic test so that a desirable bacterial count can be obtained in the fresh egg powder. Furthermore, DeBord (1925) has shown that when powder is prepared from eggs with a high bacterial count in the liquid state, rancidity develops sooner; and bacterial counts are higher in the stored product than when eggs of good quality are used. It is also recognized that dehydration of whole liquid eggs is largely responsible for a sharp reduction in the total bacterial count in the first few days of storage.

Though it would appear that the study of bacteria present in egg powder stored for considerable time would bear very little relation to the quality of the product before processing, some information of value

[1] This work has been done in co-operation with the Departments of Poultry Husbandry and Agricultural Chemistry of the Purdue Agricultural Experiment Station, and the Food Subsistence Research Laboratory of the Quartermaster Corps, U. S. Army. The author wishes to take this opportunity to acknowledge his indebtedness to Dr. B. B. Bohren who secured the samples and made several constructive suggestions as the research work progressed.

might be obtained in regard to the resistant types which survive the drying. The methods used by the bacteriologist in cultivating the weakly viable and/or dormant cells in a desiccated food product might be selected more appropriately if more were known about the genera present in this product. This investigation emphasized the bacteriology of stored, dried eggs, since it seemed that more information was needed regarding the influence, if any, of the packaging and the compression of the powder on the bacterial flora, the bacteriological methods used to determine the microbic population, and the effect of storage temperature and time on the genera of bacteria present.

EXPERIMENTAL PROCEDURE

Samples: The samples of spray-dried, whole-egg powder were supplied by the Quartermaster Corps of the Army. The eggs were processed and packaged from the same lot of powder on April 24, 1943. They were placed in storage on June 18, 1943. Samples of loose-pack egg powder in a greaseproof carton, a greaseproof carton plus a carbon liner, and a tin can were placed in rooms held at 0, 10, 20, and 37° C. (32, 50, 68, and 98.6° F.). Similar packages and temperatures of storage were employed for the compressed egg powder, except that no compressed eggs were stored in tin cans.

Plating Methods for Total Counts: The methods recommended for plating in *Standard Methods for the Examination of Dairy Products,* Part II, F, Eighth edition (1941), were followed, except that a yeast-water agar was the second plating medium instead of standard-nutrient agar as used for milk analysis. This medium had the same composition as the tryptone-glucose agar, except that a one per cent concentration of fresh yeast infusion was added—Fred and Waksman (1928). The total bacterial count for the powder in each of the several types of packages was determined immediately before placing them in storage, even though all samples were obtained from the same lot of spray-dried, whole-egg powder. All platings were made in duplicate and the counts of these plates were averaged and expressed as the number of bacteria per gram of powder. The plates were incubated at 20, 32, and 37°C., as recommended; however, only the last two incubation temperatures were retained in subsequent experimentation. As reported by Thistle, Pearce, and Gibbons (1943), plates incubated at 37°C. were found to give results similar to those incubated at 20°C. for seven days. Total counts were made on the "initial" samples, at one- and two-month intervals on some samples and at the three-month interval on all samples. No coliforms, hemophiles, anaerobes, or fungi were obtained from the samples when first received, so plates were not made for the determination of these groups thereafter.

Isolations: The isolation of bacteria from the stored samples for identification purposes were made from the colonies on yeast-water agar incubated at 32°C. The yeast-water agar slant cultures were incubated at 32°C. until good growth appeared and then transfers were made to appropriate differential media. The importance of using the yeast water in the tryptone-glucose agar was not fully appreciated until the results were analyzed. As the results will indicate, it was fortuitous that this

technique was employed from the beginning of the experiment. Bergey's "Manual of Determinative Bacteriology" (1939) was used in the final identification of the isolations.

RESULTS

Bacterial Counts: The total bacterial counts for the various types of packaging with the two kinds of powder are summarized (Table 1); the

TABLE 1

Total Bacterial Count

Sample	Package and numbering	Initial bacterial count	After 3 months' storage			
			1 S.M.,[1] 32°C.	2 S.M., 37°C.	3 Y.W.,[1] 32°C.	4 Y.W., 37°C.
A. Compressed egg powder	Greaseproof 1a[2]	500	350	0	900	0
	Carbon liner 2a	1,500	480	0	560	0
	Greaseproof 1b	500	300	0	550	0
	Carbon liner 2b	1,500	450	0	1,000	0
	Greaseproof 1c	500	TFC[3]	0	TFC	0
	Carbon liner 2c	1,500	195	0	750	0
	Greaseproof 1d	500	TFC	0	TFC	0
	Carbon liner 2d	1,500	TFC	0	TFC	0
B. Loose-pack egg powder	Tin can 3a	1,050	770	0	1,050	0
	Greaseproof 4a	2,150	850	0	1,300	0
	Carbon liner 5a	1,100	550	0	1,000	0
	Tin can 3b	1,050	550	0	1,500	0
	Greaseproof 4b	2,150	1,150	0	2,000	0
	Carbon liner 5b	1,100	650	0	1,000	0
	Tin can 3c	1,050	350	0	270	0
	Greaseproof 4c	2,150	200	0	210	0
	Carbon liner 5c	1,100	350	0	900	0
	Tin can 3d	1,050	TFC	0	TFC	0
	Greaseproof 4d	2,150	TFC	0	TFC	0
	Carbon liner 5d	1,100	TFC	0	TFC	0

[1] S. M. = standard-methods medium; Y. W. = yeast-water agar. [2] Small letters indicate storage temperature of the powder: a = 0°C.; b = 10°C.; c = 20°C.; d = 37°C. [3] A TFC count was recorded when a 1-10 dilution of the sample gave fewer than 10 colonies per plate.

different counts obtained with the two kinds of plating media and the different temperatures of incubation of these plates are also indicated. It will be noted that the initial bacterial count of the powder was comparatively low. Two factors may have been responsible for this finding: (1) the Federal specifications of fresh, dried, whole-egg powder required that total bacterial counts must be below 150,000 per gram, and (2) the powder had been made and stored for 55 days before the platings. The possible effect of this period of time with relation to the holding of the product and the bacterial count has been indicated above.

By comparing Columns 2 and 4 it is evident that after three months of storage both the compressed and loose-pack egg powder were sterile, as determined by the incubation of plates on standard-methods medium (glucose-tryptone agar) and the glucose tryptone-yeast water agar at 37°C. Higher bacterial counts were obtained with the yeast-water medium than with the standard-methods medium when the plates were incubated at 32°C. (Columns 1 and 3). This observation is in agreement with Gib-

bons and Fulton (1943), who obtained significantly different total counts when plates were incubated at 32°C. This finding indicates that many weakly viable bacterial cells were present and that the yeast-water medium provided the necessary nutrients for colonies to develop. It also indicates the desirability of using a medium recognized as being rich in essential nutrients before a sample of stored egg powder is considered to be sterile.

Essentially, the same relationships between total counts, media, and temperature of incubation of the plates were observed when platings were made at one and two months after storage of the samples.

Data on the possible influence of packaging and storage temperature on the total bacterial counts (Table 2) show that as the temperature of

TABLE 2

Total Bacterial Counts Correlated With Packaging and Storage Temperatures

Conditions of storage	Compressed egg powder		Loose-pack egg powder		
	Greaseproof carton [1]	Carton with liner [1]	Tin can	Greaseproof carton	Carton with liner
(Initial count)	500[2]	1,500	1,050	2,150	1,100
0°C.(32°F.) for 3 mo.	900	560	1,050	1,300	1,000
10°C.(50°F.) for 3 mo.	550	1,000	1,500	2,000	1,000
20°C.(68°F.) for 3 mo.	TFC[3]	750	270	210	900
37°C.(98.6°F.) for 3 mo.	TFC	TFC	TFC	TFC	TFC

[1] Greaseproof and carbon liner packages as employed by the Q.M.C. of the Army. [2] Numbers represent the bacterial count per gram of sample as determined on standard-methods medium; incubation of the plates at 32°C.(89.6°F.) for three days. [3] A TFC count was recorded when a 1-10 dilution of the sample gave fewer than 10 colonies per plate.

storage is increased the total bacterial count decreases. After three months of storage at 37°C. the reduction in total numbers is very sharp; however, the differences are not as great when samples are kept at 10 and 20°C. Storage of the powder at 0°C. resulted in very little difference in bacterial count. Gibbons and Fulton (1943) studied the influence of cooling temperature of the powder on bacterial flora and stated: "It is therefore evident that if the powder were cooled rapidly enough to preserve quality the majority of the organisms surviving the drying process would also be preserved." Similarly, these results indicate that if the product is stored at a very low temperature many more bacteria survive than if it is held at a higher temperature. Essentially the same relationship was detected when platings were made with yeast-water agar.

Apparently the compression of the egg powder reduced the total counts; however, the change was not very great. The type of packaging also had little effect upon the counts obtained; as the temperature of storage increased, there was a loss in total bacteria irrespective of the package type.

Isolations: A total of 86 isolations was made from the yeast-water agar plates. It is doubtful that if standard-methods medium had been used as a source of isolations a representative flora of the bacteria present in stored, dried eggs would have been obtained. The data may have been influenced also by the sampling factor. Obviously, when low bacterial counts are obtained in the stored product, such as those recorded previ-

ously, there is always a question of whether more generic groups would have been represented in the isolations had a larger sample been used than the 11 grams indicated in *Standard Methods.* Eighteeen primary isolations, in addition to the 84 referred to previously, were made from the yeast-water medium, but they could not be subcultured despite all efforts to make them grow. This experience suggests that factors other than a medium rich in vitamins need to be studied before more of the weakly viable cells in stored egg powder can be propagated successfully.

TABLE 3

Occurrence of Bacterial Genera in Stored, Dried Egg Powder

Sample	Initial genera in the powder	Storage period		
		1 month	2 months	3 months
A. Compressed egg powder	*B. subtilis* *M. saccatus* *M. freundreichii* *M. flavescens* *M. cinnebarius* *Bact. linens*	*B. subtilis* (d)[1] *Flavo. aurantiacum* (d) *B. ubicuitarius* (d) *Bacillus sp.* *B. tumescens* (d) *B. mesentericus* (d) *Bact. parvulum* (d)	*B. subtilis* (d) *B. mesentericus* (d) *B. simplex* (d)	*B. subtilis* (d) *Bact. linens* (a) *M. candidus* (b) *Sar. ventriculi* (b) *Bact. globiforme* (e)
B. Loose-pack egg powder	*Flavo. radiatum* *Flavo. fulvum* *Flavo. devorans* *M. freundreichii* *M. luteus* *Actinomyces sp.* *M. cinnebarius* *B. subtilis* *B. mesentericus*	*Actinomyces* sp. (d) *B. subtilis* (d) *B. ubicuitarius* (d) *Bact. parvulum* (d) *Ach. litoralie* (d)	*Actinomyces* sp. (d) *B. subtilis* (d) *Flavo. acetylicum* (d)	*B. subtilis* (a,b,c,d) *B. mesentericus* (b) *Bact. linens* (a,b,c) *Bact. globiforme* (a,c) *Flavo. aurantiacum* (a) *Flavo. rhenii* (b) *M. candidus* (b,c) *M. saccatus* (b) *Sar. lutea* (c)

[1] Small letters indicate storage temperature of the powder: a = 0°C.; b = 10°C.; c = 20°C.; d = 37°C.

The taxonomic designations of the bacteria present in the stored, dried egg powder (Table 3) show 30 different species of bacteria distributed among seven genera, as follows: *Bacillus*—7; *Micrococcus*—8; *Flavobacterium*—7; *Bacterium*—3; *Sarcina*—2; *Achromobacter*—1; *Actinomyces*—1; *Leuconostoc*—1.

It is very apparent that organisms of the *Bacillus* genus withstood the storage conditions better than organisms of any other genus. This result was expected because of the resistance of spores of the bacteria and the reports of DeBord (1925) and Stuart, Hall, and Dicks (1942). DeBord suggested that since the total count of the product made from good eggs remained practically stationary for 10 months, the counts obtained were an enumeration of the viable spores. He did not classify the species of spore-formers in the stored-egg samples, indicating that "most of them could not be identified absolutely." Some atypical reactions were obtained with certain strains isolated in this study when grown in litmus milk; however, other strongly positive morphological and physiological characteristics per-

mitted the allocation of the species. By reading from left to right (Table 3) it will be noted that the bacilli were isolated from both the compressed and the loose-pack egg powder at every interval when platings were made. It will be recalled from the total plate counts recorded earlier in this paper that the plates made with standard-methods medium, using inocula from the samples stored at 37°C. for three months, were sterile. However, the bacilli present in the samples were isolated from yeast-water medium, which suggests that even the organisms of this genus were reduced in viability by the experimental conditions but not to the point of failure to propagate themselves when suitable nutrients were present in the plating medium.

The micrococci and flavobacteria did not withstand the storage conditions very long; however, a few species were isolated after three months' storage at temperatures lower than 37°C. (Note the frequency of a, b, and c designations after the species in the right-hand column.) Why so many more species were detected in the samples plated after three months of storage than were found in the initial platings may be correlated with the adequacy of sampling.

Since *Escherichia, Aerobacter, Proteus, Alkaligenes,* and *Pseudomonas* were not found in the platings made 55 days after the packaging of the samples, it seems probable that the genera of bacteria frequently found associated with fresh eggs—Haines (1939) and Funk (1943)—do not survive very long in this product. This observation is in agreement with DeBord (1925) and Stuart, Hall, and Dicks (1942).

Most of the successful isolations obtained from the powder stored for three months were made from the samples kept at 0, 10, and 20°C. This observation indicates that as the quality of egg powder is preserved at lower storage temperatures the viability of the bacteria is likewise maintained.

No correlation could be made regarding the packaging of the powder and the isolations.

SUMMARY

The bacterial flora of spray-dried egg powder is influenced profoundly when stored at high temperatures. The total number of organisms per gram of sample is reduced as the storage temperature is increased; conversely, when the temperature is lowered higher counts are obtained and a more diversified bacterial flora can be detected. Organisms of the genus *Bacillus* predominate in spray-dried egg powder when kept for three months, irrespective of the temperature of storage.

For the isolation and determination of total counts of stored, dried egg powder, yeast-water (one part) added to glucose-tryptone agar (nine parts), recommended in *Standard Methods for the Examination of Dairy Products,* is a suitable medium. The data supports the findings of Gibbons and Fulton (1943) that higher counts are obtained when the plating media are incubated at 32°C. than at 37°C.

Storage of spray-dried, whole-egg powder in tin cans, greaseproof cartons, or greaseproof cartons plus carbon liners does not affect the total bacterial counts or the bacterial flora appreciably. Compression of the egg powder may reduce the total count slightly but appears to have little

effect upon the survival of the different species of bacteria in the sample.

In the present study eight different genera were detected in the bacterial population of stored, spray-dried, whole-egg powder. The detection of their presence was possible only when a medium rich in nutritive substances was employed. Other primary isolations were made but the conditions necessary for the continued propagation of these weakly viable strains requires further study. Obviously, statements relative to the sterility of stored, dried egg powder must be made with caution and with due regard for the methods of detecting the weakly viable genera which may be present in the product.

CONCLUSIONS

The data indicate that as the storage temperature of spray-dried, whole-egg powder is increased, the total bacterial count is decreased with time.

Yeast-water (one part) added to the glucose-tryptone agar (nine parts), recommended in *Standard Methods for the Examination of Dairy Products*, is a desirable medium for determining the total bacterial counts of stored, spray-dried, whole-egg powder.

The bacteria found most frequently in stored egg powder belong to the genus *Bacillus*. Their presence, however, may not be detected unless suitable plating media are employed.

An incubation temperature of 32°C. gives much higher total bacterial counts and more successful isolations from stored, spray-dried, whole-egg powder than 37°C.

Compression of spray-dried, whole-egg powder may reduce the total bacterial count slightly but has no noticeable influence on the genera surviving in the stored samples.

The types of packages employed in this study did not influence the bacterial flora of the stored samples.

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